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Contents for December, 1937

THE STREPTOCOCCI

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When one attempts a review of the streptococci, within reasonable limits, he must at once proscribe many interesting and important phases of the subject and thus delimit the field of discussion At the outset, therefore, it should be understood that the matter to be considered is that which appears to contribute to a better understanding of the relationships within the genus *Streptococcus*, the units or component parts being those somewhat homogeneous groups, types, or species which make up the whole, a "species," necessarily a nebulous concept when applied to bacteria, being defined along somewhat time-honored lines Whatever may be the proper species concept with respect to streptococci, it is probably safe to assume that the lines of demarcation fall somewhere between the ultra-conservative "*Streptococcus hemolyticus*" and "*Streptococcus viridans*" stage, and the hair-splitting divisions which have been set up by some taxonomically-minded bacteriologists

So limited, the scope of the proposed discussion becomes somewhat more clear The technical importances of the streptococci as agents of disease and in industry are, except incidentally, beside the point, the voluminous literature on the nutrition, growth, and general physiology of the streptococci cannot be considered, except for those phases which contribute to a systematic synthesis, serological methods which cut group or species lines are included as tools, but the immunological aspects of the streptococci are not reviewed Nor is variation, as such, considered, this phase of the streptococcus problem, however, has been included in the comprehensive reviews by Hadley (1927, 1937)

Without entering into an elementary definition of the genus

Streptococcus, it may be noted that this review is limited to forms which are bile-insoluble, which do not produce carbon dioxide, nor large amounts of volatile acids and other volatile compounds, from fermentable carbohydrates, which produce lactic acid, principally or exclusively of the dextro-rotatory form, as the dominant product of carbohydrate fermentation, and which are facultative with respect to oxygen requirements By definition, this eliminates the members of the genus *Leuconostoc*, the pneumococci, and the anaerobic streptococci The exclusion of the latter may or may not be justified in the light of present knowledge it is probable that some true streptococci, in a physiologic sense, are anaerobic, but those anaerobes which produce gas and putrefactive odors would appear likely to prove as distinct physiologically from the streptococci as are the pneumococci or the members of the genus *Leuconostoc*

As this introduction is written after the completion of the body of the text, it is quite simple to predict the outcome As much material as possible is put in tabular form and this is supplemented with additional descriptive matter, more or less pertinent from the standpoint of taxonomy, in the discussions of the individual groups or species It is of course impossible to include more than a very small fraction of the literature on streptococci, even though limited to those papers which bear, in one way or another, upon the taxonomic aspects The references actually cited are those which seemed to have pertinence as the text developed, but in addition to an incomplete bibliography the choice of selections could doubtless be improved But one point should be kept especially in mind were the selection of citations faultless for present purposes, much of the most distinguished and important work on the streptococci would not be touched As a review, not a review of reviews, this paper makes no attempt to discuss the many admirable classifications of the streptococci which have been made in the past

Attention has not been given to the numerous streptococci which have been named as "new" species without adequate descriptions to differentiate them unequivocally from other types On the other hand, it should be recognized that the

species which are now fairly clearly defined represent only a portion, perhaps a very small fraction, of those which actually exist In the case of the hemolytic streptococci, the findings with the Lancefield serological technique, which have already brought several new groups to light, point strongly toward such a view Nearly all workers with this method have reported a few "unidentified" hemolytic streptococci—in most cases probably strains of unknown species of relatively rare occurrence in the sources commonly explored by bacteriologists For the most part, the known species of streptococci are those which have brought themselves clearly to our attention as the agents of disease or as more or less dominant organisms in familiar habitats

METHODS

Progress in the identification and classification of streptococci has been made only as improved methods have been brought to bear on these problems Although a detailed consideration of methods might appear appropriate, space does not permit more than a mere mention of the tests employed, with citations, so far as possible, of the originators of the various techniques The tests used in the tables of this review are those which have had wide enough application to the various groups of streptococci so that their general significance is reasonably clear Other special methods which have value in connection with certain types are mentioned in the text It is doubtless true that many valuable methods have been suggested for the study of the streptococci, which have not been further exploited, but this phase of the subject is quite outside of the scope of the present discussion

The original use of blood agar for determining hemolytic properties among the streptococci is of course to be credited to Schottmuller (1903), and Gordon (1905a, 1905b) was certainly the first to apply the fermentation tests in a broad and systematic way In the case of some old but useful methods such as the liquefaction of gelatin, action on milk, and reducing properties, it is not possible to give credit to specific workers,

as these tests had long been in use for studying bacteria and their application to the streptococci was a gradual development and somewhat a matter of course It should be remembered that the ability to curdle milk and to reduce neutral red were among the original "Gordon's tests," these two reactions being chosen by Gordon along with certain fermentation tests as having especial value for the study of streptococci Although little employed in recent years, the ability to coagulate milk was used by all the older students of the streptococci and was considered by them to have distinct value as a differential test In some cases the action of streptococci on milk gives information of at least supplementary value, in the case of the lactose-fermenting streptococci, the information obtained by this simple test has much the same value as the determination of the final pH in glucose broth cultures As the use of litmus milk in the study of bacteria is very old, it is impossible to tell just when use was first made of reducing action in the differentiation of streptococci, but it is certain that the value of such tests was recognized before Gordon's systematic application of neutral red MacCallum and Hastings (1899) described in detail the strong reducing action of the organism which now goes under the name of *Streptococcus zymogenes*, as revealed by its ability to reduce litmus in milk cultures before the milk was curdled, and through the years strong reducing action has been looked upon as an especially marked characteristic of the lactic-acid streptococci, and, to a slightly lesser degree, of the enterococci Tests for the reducing ability of streptococci have considerable utility, and this value could probably be increased with some improvements in technique and the introduction of additional simple tests for this purpose Litmus milk as a test of reducing ability of streptococci has been applied very loosely by most workers, no consideration being given to whether the litmus is reduced before or after the milk is curdled, whereas a few workers have insisted that the vital point was whether or not reduction preceded curdling Recent studies have shown that the reduction of litmus in milk cultures before curdling is correlated with a low reduction potential, whereas the reduction which takes

place after curdling appears to have little significance inasmuch as such reduction may largely be the result of the action of the milk itself when convection currents are stopped by the formation of the curd (Knaysi and Sherman, 1937)

In the tabular material used in this paper, action on blood is limited to the true beta hemolysis as revealed in blood agar and to "non-hemolysis," including the various gradations from the gamma to the alpha reaction Brown (1919, 1937) has accurately developed the use of blood agar in the study of streptococci and has shown the value of greater refinement in its application, but in considering the streptococci as a whole it is difficult, if not impossible at the present time, to draw accurate lines between the species of non-hemolytic streptococci based upon their action on blood agar In such species as *Streptococcus salivarius*, *Streptococcus bovis*, *Streptococcus lactis* and *Streptococcus fecalis* different strains are well known to give reactions ranging from the gamma to the typical alpha type, and when consideration is given to such observations as those which have been made by Clawson (1920), Oppenheim (1920), Hagan (1925) and Gordon (1933), such variation among diverse strains of the same species would seem entirely logical No attempt is made to use the so-called "soluble hemolysin" test Todd (1934) has shown that with the application of appropriate methods all of the hemolytic streptococci studied by him produced soluble hemolysin Todd tested representatives of the Lancefield groups A, B, C, D and E, and Long and Bliss (1937a) have extended these observations to members of other Lancefield groups Hare and Maxted (1935) have called attention to the fact that, when tested by the conventional methods, the term "pseudohemolytic streptococci" may be applied with equal justice to some strains of several of the various hemolytic species

A number of valuable methods for the study of streptococci were introduced by Ayers and his several able associates The thermal resistance of streptococci was systematically studied by Ayers and Johnson (1914) in connection with the ability of certain types to survive the pasteurization of milk, and heat tolerance was later utilized as a significant physiological char-

acteristic in the differentiation of streptococci (Ayers, Johnson and Davis, 1918) Orla-Jensen (1919) applied thermal death point studies to a large number of types of streptococci in his investigation of the lactic-acid bacteria, and Houston and McCloy (1916) and Dible (1921) made use of thermal resistance as an especial characteristic of the enterococci The final pH attained in glucose broth cultures (Ayers, 1916, Ayers, Johnson and Davis, 1918) has proved of substantial merit and has been widely applied by workers on the streptococci The ability of certain streptococci to hydrolyze sodium hippurate was discovered by Ayers and Rupp (1922) The application of this reaction, for the specific purpose for which it was recommended by them, has proved to be one of the outstanding physiological tests which have been used in the differentiation of streptococci The *production of ammonia* from 4 per cent peptone by streptococci was recommended by Ayers, Rupp and Mudge (1921) as a useful differential test, and this method was fruitfully applied by these investigators in their subsequent studies As ammonia production from peptone has been used by only a few investigators in recent years, it cannot be said just how much general utility it has in the study of the streptococci However, it appears to be distinctly valuable in connection with the "viridans" types, the better known members of which do not produce ammonia, and it also has a value in the study of the lactic-acid streptococci

The uses of the temperature limits of growth and methylene-blue tolerance of streptococci were introduced by Sherman and Albus (1918) Tolerance to sodium chloride and the *alkaline pH limits of growth* (Sherman, 1921) have been used by a few workers for years in the differentiation of *Lactobacillus bulgaricus* from certain closely related lactobacilli, but these tests have only recently been systematically applied, at different levels, to the study of streptococci (Sherman and Stark, 1934, Sherman, Mauer and Stark, 1937, Sherman, Stark and Mauer, 1937, Yawger and Sherman, 1937b) The methylene-blue milk test has been used in a number of ways The work of Ayers and his associates showed that while the test as applied by

Sherman and Albus worked well in differentiating such sensitive types as *Streptococcus pyogenes* and *Streptococcus mastitidis*, on the one hand, from such tolerant forms as *Streptococcus lactis* and *Streptococcus fecalis* on the other, it had no special value when applied to miscellaneous non-hemolytic streptococci such as *Streptococcus bovis* Avery (1929a) tried methylene blue in three different concentrations and concluded that a 1 5,000 solution in skimmed milk has a value in differentiating certain tolerant hemolytic streptococci found in dairy products from the human and bovine pathogenic types, which were inhibited Avery (1929b) did not find the test as used by him of value when applied to the non-hemolytic streptococci Edwards (1933) has used, with fruitful results, a dilute solution of methylene blue in a broth medium for differentiating the hemolytic streptococci belonging to the Lancefield group C Recently it has been shown that if the concentration of methylene blue in skimmed milk is increased to 0 1 per cent, the test has some value in its application to the streptococci as a whole, all known streptococci appear to be inhibited except the members of the lactic and enterococcus groups (Sherman, Mauer and Stark, 1937, Sherman, Stark and Mauer, 1937, Sherman and Wing, 1937, Yawger and Sherman, 1937b)

Some streptococci have much more tolerance for bile than do others, and bile media have been fairly extensively used in the differentiation of these organisms The enterococci show outstanding tolerance to bile, but this characteristic appears to be shared, at least to a considerable degree, by a number of other streptococci such as *Streptococcus bovis*, *Streptococcus lactis* and *Streptococcus mastitidis* The origin of the use of bile in the study of streptococci is not clear More than twenty-five years ago, bile was used as a selective medium for the detection of fecal streptococci in milk (Kinyoun and Dieter, 1912) A little later it was observed that the mastitis streptococcus also had considerable tolerance for bile and that the test had some value for the detection of this organism in milk coming from infected udders (Rogers, Clark and Evans, 1916) Because of the known tolerance of the mastitis streptococcus to bile, Sherman

and Albus (1918) used a bile medium in an attempt to differentiate this organism from the lactic-acid streptococcus, but found that *Streptococcus lactis* was also tolerant to bile, in fact even more so than the udder streptococci under the conditions employed by them These facts have been rediscovered and put to use more systematically in the study of streptococci in recent years Weissenbach (1918) observed the tolerance of the enterococci to bile and recommended a bile medium for the differentiation of these streptococci, Meyer and Schonfeld (1926) again noted the bile tolerance of *Streptococcus lactis*, and only recently the greater bile tolerance of *Streptococcus mastitidis*, as compared with some of the other closely related hemolytic streptococci, has again come to light Recent investigators of the hemolytic streptococci have employed with some success the bile-blood-agar medium of Belenky and Popowa (1929) The hemolytic enterococci and *Streptococcus mastitidis* grow on this medium whereas the representatives of the other hemolytic species usually do not, whether or not this medium has much value in its application to the streptococci as a whole has not been determined Houston (1934) states that the ability to grow in pure bile with the production of long chains is a valuable characteristic for the identification of enterococci This suggestion is worthy of systematic investigation, but it appears from Houston's paper that some of his organisms (the "Bargen streptococcus") were probably related to *Streptococcus bovis* rather than being true enterococci, and it should be recalled that bile media were long ago shown to induce chain formation in *Streptococcus lactis* (Sherman and Albus, 1918) Although of limited usefulness, bile tolerance tests have proved valuable for certain specific purposes in the study of streptococci and it is probable that systematic investigations would reveal further special applications Safford (1937), for example, has obtained preliminary results which indicate that the greater bile tolerance of *Streptococcus bovis* may be of value as another means of differentiating this organism from *Streptococcus salivarius*

The ability of streptococci to split esculin has been curiously mixed with bile tolerance Stemming back to the use of an escu-

lin-bile-salts medium for colon bacteria by Harrison and Vanderleck (1909), many workers have studied the ability of streptococci to hydrolyze esculin in media containing bile salts. Obviously, the ability to attack esculin is one thing and the ability to grow in the presence of bile is quite another. This fact has doubtless been recognized by many workers and some of them (Weatherall and Dible, 1929, S. J. Edwards, 1932) have specifically called attention to the fact that the method as generally used is a test for bile tolerance rather than for the ability to attack esculin. The data given on esculin in this review are taken from those investigators who have applied it in the absence of bile in order to determine whether or not the streptococci could actually attack it. As esculin is very generally attacked by streptococci under favorable conditions, it does not appear to have much differential value, but the apparently consistent inability of a few types such as *Streptococcus mastitidis* and *Streptococcus thermophilus* to attack it gives esculin a limited value.

The use of starch as a fermentation test, that is, a test for the production of acid from starch, has long been used by some workers with the streptococci, but has generally been recognized as an unsatisfactory method when employed in this way. Another old method in the study of bacteria is a test for their ability to hydrolyze starch in an agar medium. It is probable that this test was applied to the streptococci by earlier workers, but its systematic application is of more recent origin. Andrewes (1930) found only 20 of 140 hemolytic streptococci from human infections able to hydrolyze starch, and only a few strains of the non-hemolytic streptococci tested had this ability. This method was also revived by Sherman and Stark (1931) who found it to have especial value in the identification of *Streptococcus bovis*, which actively hydrolyzes starch, and the test has been widely applied by these workers and their associates in the study of streptococci, especially the non-hemolytic types. As the enterococci and the lactic-acid streptococci do not hydrolyze starch, and this property is found in only certain members of the viridans group, the ability to hydrolyze starch has considerable value in the study of non-hemolytic streptococci.

Its value in connection with the hemolytic streptococci is not clear, but from the limited information at hand it appears that starch is more frequently attacked by the hemolytic types At any rate, the starch-agar test presents a method which deserves to be carefully investigated

The discovery of the lytic action on human fibrin by *Streptococcus pyogenes* and certain other types (Tillett and Garner, 1933), together with the further clarification of this phenomenon (Garner and Tillett, 1934a, 1934b), constitutes a brilliant contribution to the knowledge of streptococci and furnishes a valuable addition to the methods of studying these organisms Some cultures of *Streptococcus pyogenes* are not strongly fibrinolytic, but such strains are relatively rare, it is also true that occasional strains of various species of hemolytic streptococci may show slow fibrinolysis when the tubes are incubated at 37°C for 24 hours, it is further true that if the technique is modified in certain ways (Neter and Witebsky, 1936) various streptococci may show some action on fibrin, but if the technique of Tillett and Garner is adhered to, and arbitrary distinction is drawn between rapid fibrinolysis and weak or slow digestion, the only hemolytic streptococci which are known to be actively fibrinolytic are *Streptococcus pyogenes* (Lancefield group A) and certain types belonging to the Lancefield groups C and G This valuable test perhaps needs further standardization in order to bring out to the fullest extent its differential potentialities, but as it now stands it is not only an extremely useful reaction in the classification of the streptococci, but valuable as a presumptive test for those which are of most importance as agents of human disease The conclusion of Davis and Guzdar (1936) that this test did not have much value in *differentiating* the streptococci which are most important as human pathogens (Lancefield group A) appears to have been drawn from a faulty perspective It so happened that the collection of hemolytic streptococci with which these investigators worked was made up entirely of representatives of the Lancefield groups A, C and G—their organisms, in addition to group A, representing the only other groups which are known to contain actively fibrinoly-

tic members In the tables contained in this paper, positive fibrinolysis is limited, arbitrarily, to those organisms which cause an active and rapid lysis

The most important contribution to methods for the classification of streptococci is the serological technique of Lancefield (1933) which divides the hemolytic streptococci into groups, by means of a precipitin reaction, based on the presence in these organisms of a group-specific polysaccharide—the "C substance"—which cuts lines, though more perfectly, very closely simulating those which have long been drawn between species of streptococci The Lancefield method in no sense upsets what was previously known, but puts it on a much firmer basis and extends it, at the same time brilliantly pointing out the location of new groups or species of which bacteriologists were previously quite unaware The soluble specific substance, upon which the grouping of the hemolytic streptococci is based, was first found by Hitchcock (1924a) as a "residue antigen" and was believed by him to be common to practically all hemolytic streptococci This view was held until its group-specific nature was discovered by Lancefield (1933) Although the grouping of the hemolytic streptococci by means of the species-specific antigen may be considered, from the standpoint of the taxonomist, as her crowning achievement, this accomplishment of Lancefield developed from a series of penetrating studies on the antigenic structure of non-hemolytic and hemolytic streptococci (1925a, b, 1928a, b, c, d, e) With these other equally important phases of the immunological aspects of the streptococci we are not specifically concerned for the purposes of this review The importance of the organisms dealt with, the complexity of the problem, and the scientific and practical value of the results achieved, rank the singlehanded Lancefield work not only alone among contributions to the taxonomy of streptococci, but with few peers in the annals of modern bacteriology

It is too early to accept the Lancefield method as one giving infallible decisions, but there can be little doubt that it attains an accuracy in the classification of the hemolytic streptococci which is far beyond that heretofore possible Whether an occa-

sional strain is so lacking in group antigen as to defy identification, or whether the method may fail on rare occasions for some other reason, will be revealed as experience with the technique accumulates Although Reich (1935), in a preliminary paper, reported what he considered to be temporary changes in the Lancefield grouping of a hemolytic streptococcus, by rabbit passage, this has not been verified by full publication, whereas Gay and Clark (1937) have reported the failure to obtain such changes by passage through rabbits over a prolonged period As yet the method has not been seriously challenged, and in the meantime it has proved its worth not only in bacterial taxonomy but in the practical solution of epidemiological problems (Swift, Lancefield and Goodner 1935)

Bacteriophages have been used successfully by several investigators for certain differentiations among the streptococci and this method is now being systematically explored by Evans (Hadley and Dabney, 1926, Clark and Clark, 1927, Shwartzman, 1927, Lancefield, 1932, 1933, Evans, 1934, 1936a) Just how much value this method has in its application to the streptococci as a whole will doubtless become clear in the near future

A method which probably would have value in the systematic study of streptococci is a simple and reliable test for the possession of flagella—rather than motility, since some flagellated bacteria are not constantly motile Since the first claims of motility in streptococci by Ellis (1902a,b) there have been intermittent reports of this by other workers That motility exists in some streptococci has been proved by a number of workers, whether such motile strains represent separate species, or whether some of the ordinary streptococci are motile under certain conditions, is not yet fully clear Most of the motile streptococci have been isolated from milk, cheese or feces, and have been related to the lactic-acid streptococci (Flatzek, 1919, Schieblich, 1932) or the enterococci (Koblmuller, 1935, Levenson, 1937) Stolting (1935), however, considered his strains to be atypical forms of *Streptococcus bovis*, and found them to be different from the motile streptococcus of Schieblich (1932) A motile streptococcus isolated from milk by Dr C B Van Niel, which

has been studied in this laboratory, was found to agree in all essential physiological characteristics with *Streptococcus fecalis* and to fall in the same serological group by the Lancefield method as our stock cultures of this species

PRIMARY DIVISIONS OF THE STREPTOCOCCI

A major factor which has rendered abortive attempts to classify the streptococci as a whole has been the failure to segregate, first, in more or less homogeneous divisions, the members of the genus This is especially the case in those classifications in which the fermentation tests have constituted the major criteria on which the identifications were based Though valuable in their proper rôle of aiding in the differentiation of closely related types, the fermentation tests when applied to the streptococci as a whole, without previous subdivision by other methods, can lead only to utter confusion So far as the non-hemolytic streptococci are concerned, some such subdivision as the one here suggested is absolutely essential if any order is to be obtained in their classification, and though not so essential in the case of the hemolytic streptococci, such an approach is valuable in order to segregate at once the hemolytic members of the enterococcus division from those distantly related hemolytic groups which may for convenience be termed the "pyogenic streptococci"

It should be emphasized that there is nothing "official" about this suggested approach to the classification of the streptococci It has been in use for the past decade in the reviewer's laboratory, but it has not been used by others until very recently, and hence no claims can be made that such a division of the streptococci represents any united opinion of bacteriologists However, such a primary division does serve the important purpose of getting the related streptococci together in manageable groups, and does not in any way conflict with other methods of classification These divisions were originally based on the temperature limits of growth, supplemented by other basic characteristics, and the temperature requirements, though not

infallible, have proved to be among the most important properties of the streptococci for differential purposes (Sherman, 1937)

TABLE 1

Divisions of the streptococci

DIVISION	GROUP OR SPECIES	LANCEFIELD GROUP	HEMOLYSIS	GROWTH AT 10 C	GROWTH AT 45 C	GROWTH IN PRESENCE OF 6 5 per cent NaCl	GROWTH IN PRESENCE OF pH 9 6	GROWTH IN PRESENCE OF 0 1 per cent methylene blue	STRONG REDUCTION	SURVIVAL 60 C 30 MINUTES	NH_3 FROM PEPTONE
Pyogenic	*S pyogenes*	A	+	–	–	–	–	–	–	–	+
	S mastitidis	B	±	–	–	–	–	–	–	–	+
	S equi	C	+	–	–	–	–	–	–	–	
	"Animal pyogenes"	C	+	–	–	–	–	–	–	–	+
	The "human C"	C	+	–	–	–	–	–	–	–	+
	"Minute hemolytic"	F	+	–	–*	–	–	–	–	–	+
	Group G streptococci	G	+	–	–*	–	–	–	–	–	+
	Group E streptococci	E	+	–	–	–	–	–	–	–	+
	Group H streptococci†	H	±	–	+	–	–	–	–	±	±
Viridans	*S salivarius*		–	–	±	–	–	–	–	–	–
	S equinus		–	–	+	–	–	–	–	±	–
	S bovis		–	–	+	–	–	–	–	+	–
	Varieties of *S bovis*		–	–	+	–	–	–	–	+	–
	S thermophilus		–	–	+	–	–	–	–	+	–
Lactic	*S lactis*		–	+	–	–	–	+	+	+*	+
	S cremoris		–	+	–	–	–	+*	+*	±	–
Enterococcus	*S fecalis*		–	+	+	+	+	+	+*	+	+
	S liquefaciens		–	+	+	+	+	+	+*	+	+
	S zymogenes	D	+	+	+*	+	+	+	+*	+	+
	S durans		+	+	+*	+	+	+	–	+	+

* Indicates occasional variation from type reaction Extremely rare exceptions (e g , *Bacterium coli*, lactose –) not noted

† Group H streptococci, of doubtful status with respect to hemolysis, also fall between the "pyogenic" and "viridans" streptococci in physiological characteristics

In table 1 are given the types or species of streptococci grouped in their respective divisions The characteristics used in this table are those which are of *primary* value in separating

the divisions from one another, the further differentiation of the individual species is developed in later tables

It would seem from an inspection of table 1 that the suggested separation of the streptococci into major divisions has a logical basis Of these, the enterococci certainly represent a clearly defined division of the streptococci, the lactic streptococci represent another group which is nearly if not quite so clearly defined, less can be said for the so-called "pyogenic" and the "viridans" divisions, but they also appear to represent more or less "natural" subdivisions of the streptococci

The enterococci are markedly differentiated from the other known species of the streptococci by their combination of low minimum and high maximum temperatures of growth and by their greater tolerance to salt and alkali From most streptococci, they also differ in having high thermal death points, in being resistant to relatively concentrated solutions of methylene blue, and in usually having strong reducing action

The lactic streptococci have in common with the enterococci low minimum temperatures of growth, strong reducing action, and methylene-blue tolerance, but the lactic streptococci differ in having low maximum temperatures of growth and less tolerance to salt and alkali As will be shown later, the lactic and enterococcus streptococci are also less perfectly separated by certain fermentation tests and other characteristics

What are termed the "pyogenic" streptococci at least represent a division which conforms with past usage, as it has long been the custom to consider these species closely related They are usually hemolytic, do not grow at 10°C , and usually do not grow at 45°C , have low thermal death points, have weak reducing action, and are not tolerant to methylene blue, salt, or alkali

The "viridans" streptococci differ from the pyogenic types in not being beta hemolytic, in usually growing at 45°C , and in being unable to produce ammonia from peptone Some members of this division also have high thermal death points Like the pyogenic species, the "viridans" streptococci cannot grow at 10°C , have weak reducing power, and are not tolerant to methylene blue, salt, or alkali

In considering the natural divisions of the streptococci it is of some pertinence to consider the special status of the enterococci The term *Enterococcus* is frequently used as a separate generic designation quite outside of the genus *Streptococcus* Such names as "*Enterococcus fecalis*" and "*Enterococcus hemolyticus*" have crept into the literature Without question, the enterococci represent a clearly defined division of the streptococci, probably the most clearly marked subdivision of the whole genus But when it comes to considering the enterococci as an independent genus one may well question where, precisely, the line is to be drawn With the exception of certain tests which have only recently been systematically used in the differentiation of the enterococci, none of the properties which have been considered especially characteristic of the enterococci are peculiar to them Most certainly, the enterococci could not be established as a separate genus on the basis of morphological and general cultural characteristics, low minimum temperatures of growth, strong reducing action, and methylene-blue tolerance are shared by the lactic streptococci, high thermal death points and high maximum temperatures of growth are also characteristic of *Streptococcus bovis* and *Streptococcus thermophilus*, which are more closely related to *Streptococcus salivarius* and only rather remotely related physiologically to the enterococci, bile tolerance, at best of little value in the differentiation of streptococci, is not limited to the enterococci, as *Streptococcus mastitidis*, *Streptococcus bovis* and *Streptococcus lactis* are also tolerant to the concentrations of bile which are commonly used in the study of streptococci, and most positively a generic differentiation could not be based on habitat as many streptococci occur in the intestines and such species as *Streptococcus bovis* and *Streptococcus equinus* are just as truly intestinal forms as are the enterococci For the present at least, it would be hard to justify a generic segregation of the enterococci from the other streptococci

THE PYOGENIC STREPTOCOCCI

Putting together in one division all of the hemolytic streptococci with the exception of the hemolytic enterococci is in accord-

ance with custom In fact, the majority of those who have dealt with what they have termed "*Streptococcus hemolyticus*" have not even excluded the hemolytic enterococci from their supposedly homogeneous group The term, "pyogenic streptococci," as here used, at least limits the division to somewhat related organisms, though it must be admitted that the relationship between some of the members of this division probably is not very close

The attempted division of these streptococci into "species" is based primarily on the Lancefield serological grouping supplemented by cultural and biochemical tests There is no conflict between the serological and the physiological approaches, rather they are complementary In some of the Lancefield groups, such as A, B and F, the serological grouping and the physiological tests agree well in defining rather homogeneous species or groups On the other hand, in such groups as C and G the physiological tests are of value for the subdivision of the serological groups, most importantly, the Lancefield technique enables the cutting of certain vital lines, such as those between members of group A (*Streptococcus pyogenes*) and certain strains belonging to groups C and G, for which purpose the physiological tests thus far at hand have been unable adequately to function

Since the "species" grouping of these streptococci is based primarily on the Lancefield grouping, the review of this division must of necessity deal principally with recent work

In table 2 are presented data on the characteristics of members of the several species or groups belonging to this division

Because in recent years the hemolytic streptococci have been studied with fewer physiological tests than have the non-hemolytic forms, the assembling of adequate data on the biochemical characteristics of the pyogenic streptococci is difficult, and the data presented are woefully inadequate Much of the material presented in this table has been obtained in the reviewer's laboratory through the study of comparatively few representative cultures belonging to the various groups The compilation undoubtedly contains a number of errors as well as being incomplete, it is, however, presented without apology for what

it may be worth, and it may perhaps prove of some value in indicating a few points on which it would be desirable to extend our knowledge For example, it is possible that an extension of the test substances ordinarily used to include such compounds as glycerol, raffinose and starch might aid in lessening the confusion between *Streptococcus pyogenes* and those members of the Lancefield groups C and G from which its differentiation is not clear on the basis of the conventional tests now in vogue

TABLE 2

The pyogenic streptococci additional characteristics

GROUP OR SPECIES	LANCEFIELD GROUP	ACTIVE FIBRINOLYSIS	SODIUM HIPPURATE HYDROLYZED	STARCH HYDROLYZED	ESCULIN SPLIT	GROWTH ON 40 PER CENT BILE BLOOD AGAR	GELATIN LIQUEFIED	MILK CURDLED	FINAL pH IN GLUCOSE BROTH	ACID PRODUCED FROM										
										Arabinose	Maltose	Sucrose	Lactose	Trehalose	Raffinose	Inulin	Glycerol	Mannitol	Sorbitol	Salicin
S pyogenes	A	+*	−	−?	±	−*	−	−*	6 0–4 8	−	+	+	+*	+*	−	−	−?	±	−	±
S mastitidis	B	−	+	−	−	+	−	+*	4 8–4 2	−	+	+	+	+	−	−	−	−	−	±
S equi	C	−	−			−	−	−	5 5–4 8	−	+	+	−	−	−	−	−	−	−	+
Animal pyogenes	C	−	−	+*	+	−*	−	−	5 0–4 6	−	±	+	+	−	−	−	−	−	+	+
The human C	C	±	−	±	±	−*	−	±	5 4–4 6	−	+	+	±	+	−	−	+	−	−	±
Minute hemolytic	F	−	−	−	+	−	−	−	5 4–4 6	−	+	+	±	±	−	−	−	−	−	±
Group G streptococci	G	±	−*	±	±	±	−	±	6 0–4 6	−*	+	+	±	+*	±	−*	±	−	−	+
Group E streptococci	E	−	−	±	±	−	−	−	4 8–4 2	−	+	±	+	+	−	−	±	±	+	±
Group H streptococci	H	−	−	−	±	−	−	−	5 0–4 5	−	+	+	±	±	+	−	−	−*	−*	+

* See table 1

Streptococcus pyogenes (Lancefield Group A)

There is still a difference of opinion as to whether the most important human pathogenic streptococci, all members of the Lancefield group A, should be considered as constituting one or more than one species Without prejudice, and in accordance with what appears to be the prevailing opinion at the present time, they will be considered as belonging to one species, *Streptococcus pyogenes*

Streptococcus pyogenes (Rosenbach) has long been known "in location," so to speak, when isolated from active and severe human infections, there is little doubt that the organism which carries this name has been identified with a high percentage of

accuracy during the years Within the past fifteen years this species has become more clearly defined on the basis of its physiological characteristics but, as recent investigations have shown, in a small proportion of cases the identification can be made unequivocal only by the application of the Lancefield technique

So far as is known, *Streptococcus pyogenes* is strictly a human pathogen under natural conditions, though spontaneous infections in laboratory animals have been noted However, as was shown experimentally by Davis and Capps (1914), it is well known that the udders of cattle may become infected from human attendants, thus in turn serving as an animal source of infection with this organism among people, through the consumption of infected milk Even in this case, the infection does not appear to be self-sustaining by passing from animal to animal so as to maintain a more or less permanent source of infection Edwards (1935) studied three cultures, from baby chicks with bronchitis, which belonged to the Lancefield group A These cultures differed from the typical form of *Streptococcus pyogenes* in that they did not ferment lactose, and only one of them was actively fibrinolytic

As previously indicated, with the exception of the Lancefield serological grouping, *Streptococcus pyogenes* cannot as yet be identified with absolute certainty by means of simple physiological tests However, if this organism can be identified on the basis of what it is (as shown by serological methods) it should certainly be possible to find a way of identifying it on the basis of what it does In a sense, such distinguishing functional characteristics are now known for *Streptococcus pyogenes* its specific and unique disease relationship in man is an expression of physiological attributes not possessed by other streptococci, also, as Todd (1934) has shown and Long and Bliss (1937a) have verified, *Streptococcus pyogenes* (Lancefield Group A) is the only streptococcus among the various hemolytic groups which is known to produce an antigenic streptolysin

Although *Streptococcus pyogenes* may be readily confused with a number of other hemolytic streptococci when only the conventional physiological tests are used, this confusion can be very

largely, but not entirely, eliminated by the use of a greater variety of the imperfect tests now at hand For example, attention to such characteristics as the temperature limits of growth, action on milk, and the fermentation of raffinose, would eliminate a considerable amount of this confusion Of great value in this connection is the fibrinolytic test of Tillett and Garner Although occasional strains of *Streptococcus pyogenes* are encountered which do not have this property, the ability to lyse human fibrin is an outstanding characteristic of this organism, and if the interpretation of a positive test is limited to those streptococci which are very actively fibrinolytic, the only other streptococci known to have this action are certain types found in the Lancefield groups C and G Obviously, some additional tests to supplement fibrinolytic action are most desirable at this point The use of methylene blue as a tolerance test in a somewhat more dilute solution than that now in most general use (1 5,000) would be of some help, but would fall far short of satisfying the need Since many of the fibrinolytic members of groups C and G ferment glycerol, and some also hydrolyze starch, it would be of some value to have more explicit information on the action of *Streptococcus pyogenes* on these substances Although frequent references are made in the older literature to the fermentation of glycerol by some strains of *Streptococcus pyogenes*, information on this point since the Lancefield technique has been available is almost completely lacking With respect to the hydrolysis of starch, Andrewes (1930) found only 20 of 140 hemolytic streptococci from human sources to have this action, but one cannot be sure that these starch-hydrolyzing strains were actually members of the Lancefield group A, though some of them had come from cases of scarlet fever, puerperal sepsis and erysipelas, and three of Griffith's types of scarlet fever streptococci were represented

Streptococcus pyogenes, signifying by this term all of the members of the Lancefield group A, represents a group of streptococci showing rather diverse reactions in the fermentation tests Strains which fail to ferment lactose or salicin and others which do ferment mannitol have long been recognized, and these vari-

ations have been confirmed since the advent of the Lancefield method of grouping The trehalose and sorbitol tests have also been violated by a few authentic strains of group A streptococci (Lancefield and Hare, 1935, Davis and Guzdar, 1936) Although the present consensus of opinion is rather emphatically in favor of considering all members of group A as belonging to the one species, *Streptococcus pyogenes*, a few years ago there was a rather strong tendency toward a division of this group into several species Among the factors giving impetus to this movement may be mentioned the work of Davis (1912 and subsequent papers) on epidemic sore throat, that of Dick and Dick (1924) on scarlet fever, and the investigations of Birkhaug (1925a, b, c, 1926) on erysipelas It would seem that the evidence at this point must have been rather convincing in order to force such a sound and conservative investigator as Hektoen (1930) to the definite conclusion that "   etiologically erysipelas and scarlet fever are as distinct and different as they are clinically "

Among the different "species" which were set up within the "pyogenes group," the one which perhaps had the best claim for recognition on tangible morphological and cultural grounds was the *Streptococcus epidemicus* of Davis This organism was supposed to be especially characterized by the formation of capsules and the production of moist mucoid colonies However, it gradually became recognized that the special characteristics of this organism were properties which came within the bounds of natural variation in the streptococci, and through the works of various investigators, among which may be mentioned particularly that of Williams and Gurley (1932), *Streptococcus epidemicus* lost standing as an independent type

However, it is not yet safe to draw definite conclusions concerning the advisability of recognizing more than one species type in the "pyogenes" or Lancefield A group Evans (1937) has just put forward the claims of *Streptococcus scarlatinae* for recognition as a separate species, and the implication is given in a previous paper (1936b) that the claims of other types for species recognition will be presented in forthcoming communica-

tions As defined by Evans, *Streptococcus scarlatinae* is differentiated from *Streptococcus pyogenes* by its inability to ferment salicin Although she based the differentiation of *Streptococcus scarlatinae* on the salicin fermentation test, Dr Evans cited certain other *average* differences between *Streptococcus scarlatinae* and *Streptococcus pyogenes* which tend to support the separation made The cultures designated as *Streptococcus scarlatinae* were found to have on the average less virulence, weaker fibrinolytic action, and more tolerance to bile than did the cultures which were considered as *Streptococcus pyogenes* Though not the exclusive cause of scarlet fever, the salicin-negative strains are claimed to occur rarely in diseases other than scarlet fever or sore throat without rash

It should be noted that according to the Holman (1916) classification a salicin-non-fermenting organism which is otherwise identical with *Streptococcus pyogenes* would be designated as *Streptococcus anginosus* With some reason, it would appear, Evans rejects the name of *Streptococcus anginosus* in favor of *Streptococcus scarlatinae* which she believes should have priority for an organism of this type As will be noted later, there is also some reason to believe that the original *Streptococcus anginosus* of Andrewes and Horder (1906) was an organism which belonged quite outside of the Lancefield group A

In connection with the propriety of establishing separate species within the Lancefield group A, it is of some pertinence to consider the matter of the serological types within this group, which all agree represent only intra-species varieties Dochez, Avery and Lancefield (1919) separated four biological types of *Streptococcus pyogenes* by means of agglutination and mouse-protection tests Lancefield (1928a) also divided this group into types by means of the precipitin test based upon a type-specific protein antigen (the "M substance") which she discovered The types established by the precipitin reaction were correlated by Lancefield with the biological types mentioned above By means of his slide agglutination technique Griffith (1934) established 27 serological types of *Streptococcus pyogenes*, and stated that probably more than 30 of such types exist Al-

though it appears from later developments that a few of Griffith's types do not belong to the Lancefield group A (Hare, 1935), Pauli and Coburn (1937) have established 28 serological types of authentic group A streptococci. It has been clearly shown that the type-specific antigen may be lost in whole or in part, and within limits regained, by cultures under artificial cultivation (Lancefield, 1928a, Lancefield and Todd, 1928, Todd and Lancefield, 1928.) It would seem that the loss or acquisition of a specific protein by the cellular complex of a bacterium represents as great a degree of variation as does the loss or suppression of the ability to ferment a particular carbohydrate.

As habitat has long been considered germane to the subject of taxonomy, it is appropriate as well as of interest to review what has been learned about the natural distribution of *Streptococcus pyogenes* since its identification has been made more positive by the Lancefield grouping method. The information thus far at hand indicates that, aside from active infections, the human throat is probably the principal reservoir for the maintenance of this organism. Hare (1935) found that about one-third of the hemolytic streptococci isolated from the normal human nose and throat belonged to the Lancefield group A. Davis and Guzdar (1936) found group A hemolytic streptococci in about three per cent of the throats of 788 apparently healthy Chinese. Foote, Welch, West and Borman (1936) found that more than 20 per cent of 85 milk handlers harbored group A hemolytic streptococci in the nose or throat at least once during three months of weekly tests, five of the 20 individuals who gave positive tests for group A streptococci appeared to be constant carriers of these organisms. Lancefield and Hare (1935) rarely recovered group A streptococci from the vagina, except in cases of active infection, Hare and Maxted (1935) likewise did not find *Streptococcus pyogenes* in the feces of normal people, though it is frequently isolated from the feces in cases of throat infections such as scarlet fever, and Colebrook, Maxted and Johns (1935) failed to isolate the group A streptococcus from the perianal and perineal skin of 160 women, thus again indicating the general absence of this organism in the intestine and the birth

canal The latter investigators did, however, obtain *Streptococcus pyogenes* from the hands of seven of 181 normal individuals, but it was believed probable that the organism in these cases was derived from the respiratory tract

Streptococcus agalactiae or Streptococcus mastitidis (Lancefield Group B)

In years past, *Streptococcus mastitidis* was the name most commonly applied to the organism which is usually associated with bovine mastitis, but the tendency in recent years, both in Europe and America, has been towards the use of *Streptococcus agalactiae* It would appear from Hansen's (1935) summary of the nomenclature of this organism that though the former name has some claims for priority on the basis of its use in trinomial form, *Streptococcus agalactiae* was earlier reduced to a binomial As Hansen points out, however, both of these might be considered invalid on the basis of priority because of the generally unknown name, *Streptococcus nocardi*, which Trevisan (1889) had previously given to this organism As it can of course be reasonably argued that none of the early descriptions was complete in a modern sense, the final agreement, if any, as to the proper name will doubtless be arrived at through custom and usage

For our present purposes, it is quite impossible to review comprehensively the work on the mastitis streptococcus, but we are not primarily concerned with the historical aspects of the subject, and reference may be made to such reviews as those by Seelemann (1932), Hansen (1935), and others The useful bibliography compiled by Hansen (1934) contains references to more than one thousand papers which deal in one way or another with the subject of mastitis We shall, therefore, consider very briefly the taxonomic aspects of the organism, with especial attention to the newer facts which have been learned about it since the Lancefield method of serological grouping has been available

Although some good descriptions of the organism may be found in earlier works, the precise identity of *Streptococcus*

mastitidis may well be dated from the work of Ayers and his co-workers, who introduced new tests and clearly differentiated this organism from the *Streptococcus pyogenes* of human infections (Ayers, Johnson and Davis, 1918, Ayers and Rupp, 1922, Ayers and Mudge, 1922) These investigators correlated the production of a low pH in glucose broth, limited and variable hemolytic power, and the ability to hydrolyze sodium hippurate, in the differentiation of the mastitis streptococcus from hemolytic streptococci from human infections The ability to produce a low final pH was quickly confirmed by others (Avery and Cullen, 1919, Brown, 1920) and the sodium hippurate reaction has remained, for its specific purpose, one of the outstanding differential tests used in bacteriology

The diverse action on blood by various strains of *Streptococcus mastitidis* has been supported by the work of many investigators since Ayers and his associates, and that such variation occurs within the species has also been confirmed serologically Stableforth (1932) showed that hemolytic and non-hemolytic strains frequently were of the same serological type, and Lancefield (1934b) has reported the loss of the property of hemolysin production in a culture of *Streptococcus mastitidis* without change in immunological specificity as to group and type

The production of pigment, ranging in color from yellow to brick-red, is a rather characteristic property of *Streptococcus agalactiae*, and references to this are found in some of the early descriptions of this organism Among relatively recent investigators, Orla-Jensen (1919) mentioned the production of an orange color in casein-peptone broth containing soluble starch, as being very characteristic of his cultures of *Streptococcus mastitidis* However, all strains are not chromogenic, and Lancefield (1934b) has shown further that the property of producing pigment may be lost in laboratory cultures Another characteristic which appears to make the mastitis organism somewhat unique among the hemolytic streptococci is its apparent total inability to attack esculin (Diernhofer, 1932 Orla-Jensen 1934, Hansen, 1935)

Aside from its occurrence in bovine udders showing definite

signs of infection, the mastitis streptococcus has long been known to occur in the udders of a large proportion of cattle which appear, superficially at least, to be entirely normal (Sherman and Hastings, 1915, Evans, 1916) These observations have been confirmed by many workers, though it is a nice question just what may be considered a "normal" animal However, Hucker (1937a) has reported the isolation of *Streptococcus agalactiae* from the aseptically-removed udder tissues of cows known to be free of mastitis In some cases the organism was also obtained from the udder tissues of virgin heifers The mastitis streptococcus is not known to occur in the bovine mouth or intestine, but in view of the information which has recently come to light concerning the harboring of this organism by human beings, the possibility should be recognized that there may be other animal reservoirs for this organism, aside from the udder

Lancefield group B streptococci, physiologically and serologically entirely typical of *Streptococcus mastitidis* or *Streptococcus agalactiae*, have been isolated from a number of human sources from the nose and throat (Lancefield, 1933, Plummer, 1935, Hare, 1935), from the vagina (Lancefield and Hare, 1935), and from feces (Smith and Sherman, 1937) Although *Streptococcus agalactiae* most certainly cannot be looked upon as a human pathogen, as in the case of a number of other "non-pathogenic" streptococci, the possibility of its rare occurrence in human infections should be recognized Lancefield and Hare (1935) noted a few cases in which it seemed possible that group B streptococci were the cause of mild infections of the uterus, and Hare (1935) has since reported two cases of fatal uterine infection following childbirth

Stableforth (1932) and Lancefield (1934a) studied the serological types of *Streptococcus agalactiae* by means of agglutination and precipitin tests, each establishing three types within the group (the type-specific antigen in group B—the "S substance"—being a polysaccharide, Lancefield, 1934a) Stableforth (1937) again investigated this problem with a larger assortment of cultures and obtained a total of five types consisting of three main types, two of which contained related subtypes Stewart

(1937) studied 72 strains from Australia and found four types represented

In spite of some variability with certain tests, such as its reactions on blood and salicin, *Streptococcus agalactiae* appears to represent a clearly defined and homogeneous group of organisms Nevertheless, there can be little doubt that a number of the "species" of hemolytic streptococci which have been reported from the bovine udder have in fact been strains of *Streptococcus agalactiae* which varied slightly from type in some of the biochemical tests This is true of at least some of the strains which have been described under the name of *Streptococcus asalignus* (Frost, Gumm and Thomas, 1927) Two cultures of *Streptococcus asalignus*, kindly furnished by Dr Frost, were found to belong to the Lancefield group B, when tested serologically in our laboratory, and to be typical physiologically of *Streptococcus mastitidis* or *Streptococcus agalactiae*, though they were more strongly hemolytic than is usual for members of this species, and also failed to ferment salicin

Plastridge and Hartsell (1937) have very recently described as a new species, *Streptococcus pseudo-agalactiae*, an organism obtained from the bovine udder In biochemical reactions this organism is the same as *Streptococcus agalactiae* except that milk is usually not curdled, though sometimes coagulation takes place after incubation for seven days, also, there was frequently a slight reduction of methylene blue which was not caused by typical cultures of *Streptococcus agalactiae* Plastridge and Hartsell state "Although the *S pseudo-agalactiae* cultures were usually somewhat less hemolytic than the *S agalactiae* cultures, and the reactions of the two organisms in litmus milk and methylene blue milk were at times slightly different, none of the biochemical tests gave results which were sufficiently clear-cut for differential purposes"

Serologically, *Streptococcus pseudo-agalactiae* is believed by Plastridge and Hartsell to represent a distinct group By the Lancefield method, extracts of *Streptococcus pseudo-agalactiae* gave slight or partial reactions with both groups B and C antisera, good reactions, however, being obtained with antisera pre-

pared against cultures of *Streptococcus pseudo-agalactiae* On the other hand, extracts of typical group B organisms gave only partial or negative reactions when tested with antisera prepared against *Streptococcus pseudo-agalactiae* Another reason given by Plastridge and Hartsell for considering their organism a new species is that it appears to be less frequently associated with mastitis than does the typical *Streptococcus agalactiae*, some of the udders yielding the pseudo-agalactiae type show no laboratory evidence of mastitis, and the infection, with or without evidence of mastitis, is usually of short duration

It is too early to pass judgment on the taxonomic status of Plastridge and Hartsell's organism In view of the somewhat equivocal results, it would appear to be serologically related to *Streptococcus agalactiae*, as well as practically identical on the basis of the physiological tests Further work will doubtless show whether it should be considered only a variety or given rank as a separate species

Streptococcus equi (Lancefield Group C)

The Lancefield group C appears to contain three rather clearly defined biochemical groups which may justify, in the present state of our knowledge, consideration as separate species *Streptococcus equi*, the cause of "strangles" in horses, the "animal pyogenes" or the "animal C" type, and the "human C" streptococcus which is the characteristic group C form obtained from human sources

Streptococcus equi has been carefully studied by a good many specialists in comparative pathology, but is little known by bacteriologists generally, hence reference may best be made to the works of the authorities on this subject for a complete history of the organism Jones (1919) gave a review of the subject up to the time of his work, and among recent workers may be mentioned especially Ogura (1919), Edwards (1933, 1934, 1935, Dimock and Edwards, 1933) and Evans (1936c) *Streptococcus equi* as a scientific name is generally credited to Schutz (1888), and is so credited by Bergey (1934), but Evans (1936a) states that its use by Sand and Jensen (1888) appears to be its first use as a specific name for the organism of strangles

Those who have worked with it intimately, and should therefore be best qualified to judge, feel that *Streptococcus equi* represents a very definite and distinct physiological and clinical entity possessing characteristic and relatively constant properties It is true that there has been much disagreement about the characteristics of *Streptococcus equi* among the older workers in this field, but from recent contributions it appears probable that most of these differences of opinion were due to a confusion with other closely related animal streptococci For example, a number of investigators have reported lactose-fermenting streptococci, isolated from horses suffering from strangles, which were considered atypical strains of *Streptococcus equi* Edwards (1935) dissents from this view and states that lactose-fermenting hemolytic streptococci (the "animal pyogenes" type) are sometimes found as secondary invaders, and in a recent paper Evans (1936c) concurs in the view that such lactose-fermenting strains should not be considered *Streptococcus equi*

Its inability to ferment lactose, trehalose and sorbitol appears to serve fairly adequately for the differentiation of *Streptococcus equi* from the other group C streptococci

Streptococcus equi	(lactose −, trehalose −, sorbitol −)
"Animal pyogenes"	(lactose +, trehalose −, sorbitol +)
"Human C"	(lactose ±, trehalose +, sorbitol −)

However, its identity does not rest entirely on those grounds *Streptococcus equi* is more delicate in its nutritive requirements, very little or no growth occurring in ordinary laboratory media The "human C" type, which on the basis of its actions on lactose and sorbitol might appear very closely related to *Streptococcus equi*, shows rather striking differences when a larger number of physiological test substances are used, the "human C' streptococcus shows greater tolerance to methylene blue (Edwards 1933, 1935), is usually actively fibrinolytic, and commonly ferments glycerol

As some strains belonging to the Lancefield group A fail to ferment lactose, and a few strains do not attack trehalose, there is a possibility of confusing *Streptococcus pyogenes* and *Streptococcus equi* when the fermentation tests alone are depended upon,

although the possibility of such an error is not great However, other methods in addition to the serological grouping are available for the separation of these two organisms Following the Holman (1916) classification, in which the differentiation of *Streptococcus pyogenes* from *Streptococcus equi* rests entirely on the fermentation of lactose, many workers have reported as "*Streptococcus equi*" cultures of hemolytic streptococci from human sources, which did not ferment lactose Evans (1936c) examined a number of such lactose-negative cultures obtained from Coburn and Pauli (1932), Fisher (1933) and Plummer (1934), and found that all of them could be readily differentiated from *Streptococcus equi* on the basis of their actions on human fibrin and trehalose On the basis of the fermentation tests alone, the organism which could be most readily confused with *Streptococcus equi* is the "minute hemolytic streptococcus" of Long and Bliss (1934) This organism does not ferment sorbitol and may or may not ferment lactose and trehalose, and it resembles *Streptococcus equi* in growing poorly in ordinary laboratory media and in not lysing human fibrin These two organisms belong to entirely different serological groups, and it is likely that their relative cellular and colonial "minuteness" would prevent their confusion by one who is familiar with the two organisms Evans (1936c) reports that she has studied a number of strains of hemolytic streptococci from human sources which failed to ferment lactose, trehalose and sorbitol, "but which are otherwise very unlike *S equi*" She also states "In our collection of about 600 strains of hemolytic streptococci, which includes about 150 strains from animal disease sources and about 400 strains from human disease sources, there was no strain agreeing with *S equi* from any disease other than strangles"

Other characteristics of *Streptococcus equi* which are considered somewhat unique are its high degree of virulence for white mice combined with low, or no, virulence for rabbits and guinea pigs, and its inability in general to cause agglutinin formation when injected in animals, though some "atypical" strains may have this antigenic action (Evans, 1936c)

It is possible that some of the differential characteristics which

have been claimed for *Streptococcus equi* do not rest on sound foundations, but when consideration is given to all that is known about its serological grouping, its physiological characteristics, its virulence, its very specific connection with a certain disease, and the various other peculiarities ascribed to it, there can be little doubt that it represents a unique type which may properly be designated as a species, as species are commonly defined in bacteriology

The "Animal Pyogenes" Streptococcus (Lancefield Group C)

The hemolytic streptococcus belonging to the Lancefield group C, which is here designated for convenience as the "animal pyogenes," has only recently become clearly defined Before the work of Lancefield (1933), which established its status serologically, this type had taken rather clear form as a separate entity through the works of Ogura (1929) and Edwards (1932, 1933) More recently, the physiological and serological characteristics of this organism have been further confirmed and extended by Edwards (1934, 1935), Plummer (1934, 1935) and others

Physiologically the "animal pyogenes" is especially marked by its ability to ferment sorbitol and its inability to ferment trehalose, a combination of properties which, so far as present knowledge extends, appears to be unique among the hemolytic streptococci Also, on the basis of the rather extensive data thus far gathered, the action of this organism on trehalose and sorbitol has proved remarkably constant

All who have reviewed the literature on *Streptococcus equi* have cited early writers to the effect that the organism of strangles does not ferment lactose and sorbitol, whereas the "*Streptococcus pyogenes*" of other equine infections does ferment these two substances This observation, which is contained in early treatises on *Streptococcus equi*, is credited to Holth in what appears to be an unpublished communication before 1911 (See Jones, 1919, Edwards, 1935, and Evans, 1936c) However, it only recently became clear that the common hemolytic streptococcus of animal infections could be differentiated from the

true *Streptococcus pyogenes* of human infections by means of its action on sorbitol, though there have doubtless been valuable observations which have remained unexploited For example, Orla-Jensen (1919) called attention to the fact that the cultures of *Streptococcus pyogenes* from human sources which he studied did not ferment sorbitol, whereas the strains obtained from animal infections did attack this substance

In spite of the undoubted value of the trehalose and sorbitol tests in their application to hemolytic streptococci belonging to the Lancefield group C, it should again be emphasized that we are not yet acquainted with all of the important types of streptococci For example, Minett (1935) has reported a number of strains of hemolytic, lactose-fermenting streptococci isolated from dogs, cats and ferrets, which fermented neither trehalose nor sorbitol, but it is not known whether or not these were group C streptococci, though some of them were associated with infections in lower animals However secure present criteria may appear to be, new methods and revised definitions are apt to be necessary in connection with this and other groups of streptococci, as knowledge of the now unknown types increases

Edwards, who has studied this streptococcus most extensively, has shown that the "animal pyogenes" is remarkably constant in other characteristics, in addition to its actions on trehalose and sorbitol and its serological grouping All of his strains fermented lactose and salicin and produced capsules, whereas none of them fermented mannitol nor glycerol, none greened "chocolate agar," and all were inhibited by dilute methylene blue under the conditions of the test as applied by him It should also be recalled that Lancefield (1932, 1933) found this streptococcus to be peculiarly sensitive to a particular race of bacteriophage (Clark and Clark, 1927) which she employed Its group-specific antigen and biochemical reactions certainly make the "animal pyogenes" a distinct "species" when compared with the hemolytic streptococci outside of the Lancefield group C, and for the present at least its recognition as a definite entity of specific rank, within group C, appears justified

Hemolytic streptococci of the "animal pyogenes" type cause

a variety of infections in lower animals They sometimes occur in the bovine udder, occasionally causing mastitis, and may be frequently isolated from raw milk It is now known (Dimock and Edwards, 1933) that some of the hemolytic streptococci from milk (not associated with epidemic sore throat) which were formerly considered "*Streptococcus epidemicus*" were in fact the "animal pyogenes" type There is no evidence that this streptococcus has any significance from the standpoint of human health, and, so far as the reviewer is aware, it has not yet been isolated from human sources

The "Human C" Streptococcus (Lancefield Group C)

The hemolytic streptococcus which is here designated as the "human C" type first began to take form as a distinct entity as an organism occasionally encountered from animal sources Ogura (1929) identified this streptococcus (trehalose +, sorbitol −) as "Type B," as opposed to his "Type A" (trehalose −, sorbitol +) and *Streptococcus equi* (trehalose −, sorbitol −) As fermenting and non-fermenting strains were found with lactose, two varieties of type B were recognized B1 (lactose −) and B2 (lactose +)

Before it became clearly established through the application of the Lancefield serological grouping method, this streptococcus was given more standing as an independent type by the work of Edwards (1932, 1933) He showed that the few cultures of this organism obtained from animal sources differed from the common "animal pyogenes" type in their respective actions on trehalose and sorbitol, and that the "Type B" streptococcus (trehalose +, sorbitol −) differed in being more tolerant to methylene blue, in not producing capsules, and in the greening of "chocolate agar" On the other hand, the fermentation tests did not effectively differentiate this ("Type B") streptococcus from *Streptococcus pyogenes* of human origin, but its greater tolerance to methylene blue separated perfectly the few cultures of this type from the true *Streptococcus pyogenes* (Although a few of the "human hemolytic streptococci" were tolerant to methylene blue, Edwards later showed (1935) by means

of the precipitin reaction that these cultures actually belonged to the Lancefield group C)

After the advent of the Lancefield serological technique it became apparent that whereas the usual group C hemolytic streptococcus from animal sources (the "animal pyogenes") ferments sorbitol but not trehalose, the type obtained from human beings ferments trehalose but not sorbitol From human sources, there have now been reported some 80 or more cultures of the "human C" type of streptococcus by Lancefield and Hare (1935), Edwards (1935), Hare (1935), Plummer (1935) and Davis and Guzdar (1936), all of these strains have fermented trehalose and failed to ferment sorbitol

Its serological grouping clearly differentiates the "human C" streptococcus from *Streptococcus pyogenes* (Lancefield group A), but on the basis of physiological tests it must be admitted that confusion is still possible The possibility of such confusion is enhanced by the fact that the "human C" streptococcus is usually actively fibrinolytic However, Edwards (1935) showed that all of his cultures of *Streptococcus pyogenes* (Lancefield group A) were inhibited by methylene blue, as used by him, whereas all of his "human C" streptococci grew Edwards (1933) used a beef infusion-casein digest broth containing methylene blue in a concentration of 000025 molar This test, as employed by Edwards, has not been used by others, but Davis and Guzdar (1936), with a 1 5,000 concentration of methylene blue in sterile milk as the test medium, confirmed Edwards' findings, though the differentiation was not perfect In this connection, the ability to ferment glycerol may offer some supporting, though not perfect, evidence In his early work Edwards (1932) showed that all of his five cultures of this group C streptococcus fermented glycerol, but in his later work (1935), with a somewhat larger collection, results with glycerol were not reported Mr Niven, in this laboratory, has examined a small number of "human C" streptococcus cultures, obtained from Dr Lancefield, and has found them to ferment glycerol However, it is obvious that some new methods will have to be developed before the "human C" streptococcus can be satisfac-

torily differentiated from *Streptococcus pyogenes*, except by the Lancefield serological technique

Since both belong to serological group C, the propriety of recognizing the "human C" type as a "species" separate from the "animal pyogenes" may be questioned Their respective actions on trehalose, sorbitol, glycerol, human fibrin, and "chocolate agar," together with their differences with respect to methylene-blue tolerance and capsule formation, give a rather imposing basis for considering these two organisms as distinct types

The "human C" streptococcus has been obtained from the normal human nose and throat (Hare, 1935, Davis and Guzdar, 1936), vagina (Lancefield and Hare, 1935) and skin (Colebrook, Maxted and Johns, 1935) There is no evidence that this organism is of very much importance as a cause of human disease (Lancefield and Hare, 1935), but it appears that it may sometimes cause erysipelas (Hare, 1935) Plummer (1935) studied two cultures from puerperal fever and three from erysipelas With respect to animals, Edwards (1935) states that this streptococcus is of low virulence and, when present in severe infections, is usually associated with other streptococci

The "Minute Hemolytic Streptococcus" of Long and Bliss (*Lancefield Group F*)

The "minute hemolytic streptococcus" discovered by Long and Bliss (1934) represents a new species of streptococcus of undoubted authenticity In blood agar plates this organism produces extremely small "pin-point" colonies, frequently barely visible, but surrounded by a definite zone of true hemolysis ranging from a little more than 0 5 to about 1 5 mm after 48 hours

Long and Bliss based this new species not only on its unique morphology, colony formation, and fermentation reactions, but also reported preliminary serological investigations which showed that this organism did not belong to any of the then established Lancefield groups (A, B, C, D and E) Shortly afterward,

Lancefield and Hare (1935) identified this organism from the human vagina, and designated it as serological group F

Although the "minute hemolytic streptococcus" gives a rather characteristic "pattern" on the fermentation tests, some diversity is found among different strains on some of the more commonly used test substances Lactose, trehalose and salicin may or may not be fermented, but a vast majority of strains ferment trehalose and salicin Relatively few of the strains reported by Long and Bliss fermented lactose, whereas a majority of those reported by Hare (1935), Hare and Maxted (1935) and Lancefield and Hare (1935) did attack this substance The group F streptococcus may or may not produce "soluble hemolysin" when subjected to the conventional test (Hare and Maxted, 1935), but Long and Bliss (1937a) have shown that this substance is abundantly produced with the application of appropriate methods Aside from its serological grouping, the identity of the "minute hemolytic streptococcus" is perhaps established more firmly by its morphological and colonial characteristics than by its physiological properties In this connection, it is desirable to give a few direct quotations from Long and Bliss (1934), who have carefully studied these features of their organism

In films made from cultures in liquid medium and stained by Gram's method the organisms appear as minute cocci occurring singly, in pairs, in short chains and in small and large masses The individual coccus is one-half to two-thirds the size of the ordinary *beta* hemolytic streptococcus and it stains indifferently with Gram, some strains being strongly Gram-positive while others are Gram-negative

When the areas of hemolysis first become visible the colony cannot be distinguished by means of the unaided eye, and resort must be had to the use of the low power of an ordinary microscope At this stage of development, the colony appears as a small, finely granular, roughly circular object ranging in size from 18 to 30 microns and surrounded by a relatively large area of true *beta* type hemolysis Occasionally the colonies appear to be wrinkled and crenated or they may have a curious tetradic appearance Rarely have the colonies been oval in the primary culture By the end of 48 hours incubation they are visible to the naked eye, although, in certain instances, 96 hours of incubation

were required before they were visible In the first stages of development the ratio of the diameter of the area of the hemolysis to the diameter of the colony is roughly from 4 to 1, to 10 to 1 With further incubation this ratio decreases so that by the end of 48 hours the ratio is generally 3 or 4 to 1 Ordinary *beta* hemolytic streptococci from human sources have a ratio of 3 or 4 to 1 from the time the colonies are first visible and preserve this ratio throughout the period of incubation

Bliss (1937) has investigated the serological characteristics of the group F "minute" streptococci By means of the slide agglutination technique four serological types, within group F, were established

Long and Bliss (1934) and Long, Bliss and Walcott (1934) have isolated the "minute hemolytic streptococcus" from the throats of normal people and from those suffering with a variety of diseases, especially glomerular nephritis and rheumatic fever In a few cases the organism was recovered in pure culture from pus removed from inflamed sinuses and abscesses These investigators have been conservative in their conclusions and the importance of this streptococcus as a cause of human disease is still held to be problematical However, Bliss (1937) has referred to a paper now in press (Long and Bliss, 1937b) which reports several cases in which group F streptococci appeared to be the primary cause of disease

Regardless of its possible importance as an occasional invader of the human body, there is no doubt that the "minute hemolytic streptococcus" commonly occurs as a harmless human parasite Aside from its occurrence in the normal throat, this streptococcus has been obtained from the human vagina (Lancefield and Hare, 1935), skin (Colebrook, Maxted and Johns, 1935) and feces (Hare and Maxted, 1935)

The Streptococci Belonging to the Lancefield Group G

The streptococci of this group were discovered through the work of Long and Bliss (1934) and were noted by Lancefield and Hare (1935) as the serological group G In their paper dealing with the physiological classification of "minute hemolytic streptococci," Long and Bliss (1934) segregated as "group II"

the organisms which fermented lactose Correlated with the fermentation of lactose, in most of the cultures, was a somewhat wide zone of hemolysis surrounding the colonies in blood agar plates Preliminary studies of the antigenic structure of these organisms had shown that this type was serologically different from the other "minutes" (group F), and later studies (Bliss, 1937) showed that these somewhat larger "minutes," which showed a wider zone of hemolysis, belonged to the Lancefield group G, which group in the meantime had been established by Lancefield and Hare (1935) on the basis of cultures isolated from the human vagina

From an inspection of the characteristics ascribed to group G streptococci in table 2, it would appear that this group is made up of diverse physiological types Physiological studies of group G cultures indicate that this group, like group C, contains at least two, and more probably three, biological types which may eventually be recognized with some degree of accuracy by the use of biochemical tests In this apparently heterogeneous group there occurs one clearly defined and homogeneous physiological type which future work may indicate should be recognized as a distinct variety, or even species, within group G This type is the one which Long and Bliss (1934) included among their "minute hemolytic streptococci" As the more recent work of Bliss (1937) has shown, the members of group G which Long and Bliss had previously classified as "minute" streptococci all belong to serological type I, whereas the strains which were considered as beta hemolytic streptococci of ordinary size do not belong to this serological type

Aside from the "minute" or type I variety, the other members of the Lancefield group G appear to represent rather diverse physiological types Among these are found strains which in physiological characteristics, including strong fibrinolytic action, appear to be identical with *Streptococcus pyogenes* On the other hand, some strains belonging to group G differ widely from *Streptococcus pyogenes* in that they are able to attack a variety of substances including glycerol and starch, sometimes even fermenting weakly inulin and the pentose sugars This type, which may or

may not be fibrinolytic, also differs widely from *Streptococcus pyogenes* in being more tolerant to methylene blue, in having stronger reducing action, and in the ability of some strains to grow at 45°C

From studies made in our laboratories with cultures furnished by Drs Bliss and Lancefield, as well as additional strains isolated from human feces, it appears that the "minute" or type I (Bliss, 1937) group G organisms represent a rather clearly defined physiological variety of streptococcus Although similar to *Streptococcus pyogenes* with the conventional tests, all of the type I strains which we have thus procured have been found to ferment raffinose, curdle milk, and to be devoid of strong fibrinolytic action, thus being differentiated physiologically from *Streptococcus pyogenes* In making these statements it should be emphatically asserted that no claim is made that serologically types cut "species" lines in group G It is not certain, nor even highly probable, that all strains which have these physiological characteristics will prove to belong to type I It just so happens that, among the limited number of cultures which have been studied, the members of serological type I conform to a rather definite physiological pattern which differs from that of the non-type-I strains

It is perhaps proper to point out similarities between newly discovered organisms and types which have been previously described Attention is therefore called to the likeness of the type I group G streptococcus to the *Streptococcus anginosus* of Andrewes and Horder (1906) Before entering this discussion, however, it should be made clear that different criteria have been used in the identification of this organism In the Holman (1916) classification, *Streptococcus anginosus* is described simply as a hemolytic, lactose-fermenting streptococcus which ferments neither mannitol nor salicin *Streptococcus pyogenes* is given the same characteristics except that salicin is fermented, the differentiation of these two organisms resting wholly on the salicin reaction We are not concerned with which is the "correct" definition, only that they are different The present comparison is with the type as given by Andrewes and Horder

Andrewes and Horder (1906) described as *Streptococcus anginosus* a hemolytic streptococcus which they isolated from normal human throats, and frequently also from cases of scarlet fever and other forms of sore throat *Streptococcus anginosus* was differentiated from *Streptococcus pyogenes* by its ability to curdle milk, ferment raffinose, and produce a stronger reducing action in neutral red cultures They also noted variants from the type, some of which did not ferment raffinose, while others fermented inulin in addition to raffinose Fermenting and non fermenting strains were found with salicin, the majority failing to attack this substance There can be little doubt that *Streptococcus anginosus* as described by Andrewes and Horder represents a distinct type from *Streptococcus pyogenes*, and since the other long-established species of pathogenic, hemolytic streptococci, such as *Streptococcus mastitidis* and *Streptococcus equi*, never ferment raffinose, *Streptococcus anginosus* (Andrewes and Horder) would seem to represent an authentic type or species This organism has apparently been lost during the past thirty years The reason for this loss is not far to seek, it was caused by concentrating on a few reactions and at the same time discarding tests of merit for the portrayal of the characteristics of streptococci Hence, the selective raffinose fermentation was dropped, the old-fashioned milk test was eliminated, and tests for the relative reducing abilities of streptococci were discontinued Thus the tests which were especially advocated by Andrewes and Horder for the recognition of their newly discovered species fell by the wayside

Again it should be emphasized that aside from those organisms of the Lancefield group G which bear a relationship to *Streptococcus anginosus*, and represent an apparently closely related and homogeneous type, there occur other types which do not appear very closely related either to the *Streptococcus anginosus* type or to each other

The hemolytic streptococci belonging to the Lancefield group G are widely distributed They have been obtained from the normal human throat and nose, vagina, skin and feces Certain types belonging to this group have also been obtained from

normal animal throats and from animal infections, their occurrence in the latter being possibly only as secondary invaders (Long and Bliss, 1934, Lancefield and Hare, 1935, Hare, 1935, Hare and Maxted, 1935, Colebrook, Maxted and Johns, 1935, Davis and Guzdar, 1935) There is no present reason for believing that the group G streptococci are of much importance as potential producers of human disease Lancefield and Hare (1935) obtained one strain from a severe case of puerperal infection, but in this case there was also a heavy staphylococcus infection In the light of present information, it seems probable that group G streptococci are only of occasional, if any, importance from the standpoint of human health

The Lancefield Group E Streptococcus

The group E hemolytic streptococcus was established by Lancefield (1933) on the basis of three cultures which had been obtained from milk As only a few strains of the group E streptococcus have been serologically identified since the work of Lancefield, it is not known how commonly this type occurs, thus far, it has been reported only from the bovine udder and milk The few cultures which had been subjected to detailed study show considerable diversity in respect to the fermentation tests and the identity of this streptococcus as yet rests entirely on the Lancefield serological grouping In view of the limited knowledge of this group, Dr Lancefield's brief statement concerning it is reproduced

Group E comprised three strains isolated by Dr J H Brown from certified milk They were members of Groups 3 and 6 described by Brown, Frost, and Shaw (1926) On preliminary examination they were thought to be members of Group C of this series because one of them, K 131, showed some cross-reaction with Group C antisera and because all three were very hemolytic on blood agar plates and also markedly susceptible to streptococcus bacteriophage Further work showed that the precipitin reaction of Strain K 131 with Group C antisera was a minor one, not exhibited by the other two strains, and that antisera prepared against these three strains showed strong precipitin reactions with extracts of all three

The antiserum from one rabbit immunized with Strain K 129 showed traces of cross-reactions with almost all extracts tested This was not evident with the antiserum from another rabbit, hence it was felt that this difference had to do with an individual rabbit variation rather than with antigens contained in this strain

The cultural characteristics of this group were the following the final pH attained in dextrose broth was 4 6 to 4 8, sodium hippurate was not hydrolyzed, methylene blue milk was not reduced, growth was not obtained on bile blood agar even when the concentration of bile was only 10 per cent, both trehalose and sorbitol were fermented, and the streptococcus bacteriophage caused rapid lysis of all three strains It is not known how extensively this group is distributed, nor whether it is composed entirely of strains derived from milk, although the three classified here were isolated from certified milk

It will be noted that group E streptococci ferment both trehalose and sorbitol, which is unique among the established species of the hemolytic streptococci, outside of the enterococcus division, it should be recognized, however, that not sufficient strains have been studied to establish these properties as constant characteristics of the group It is probable that to this group belong at least a portion of the hemolytic streptococci reported from milk, which ferment both trehalose and sorbitol (Minett, 1935) Among the few strains of group E streptococci thus far studied, there have been cultures which fermented both mannitol and salicin, and others which fermented neither of these substances It is therefore likely that some of the hemolytic streptococci from milk which have been reported under the name of *Streptococcus infrequens* (Frost, Gumm and Thomas, 1927, Minett and Stableforth, 1934) were members of this group, and it is also possible that some of the strains designated as *Streptococcus asalignus* (Frost, Gumm and Thomas, 1927) were group E streptococci, though most of these were probably members of group B

The group E cultures reported by Lancefield were strongly hemolytic, but Plastridge and Hartsell (1937) have reported strains obtained from the bovine udder which they classified as weakly hemolytic An important point which supplements

the Lancefield serological classification in establishing the group E streptococcus as an authentic species, is the work of Todd (1934) which showed that the streptolysin produced by this organism has characteristic properties, its acid stability being especially outstanding

The Group H Streptococcus of Hare

By means of the Lancefield serological method Hare (1935) segregated as a new group (group H) hemolytic streptococci obtained from the human nose and throat Hare and Maxted (1935) also recovered two group H strains from human feces These cultures did not lyse human fibrin, hydrolyze sodium hippurate, grow on 40 per cent bile blood agar, nor produce "soluble hemolysin" Hare studied the fermentation reactions of his cultures with lactose, trehalose, mannitol, sorbitol and salicin All of the strains fermented salicin, twenty-four of the 25 cultures failed to ferment mannitol and sorbitol, but one culture fermented both of these substances, a majority of the cultures fermented lactose (22 +, 3 −) and trehalose (21 +, 4 −) It is evident that the members of this group show some diversity in the fermentation tests Since the group H streptococcus was discovered by Hare and the identity of the group rests on his work, it is desirable to quote Hare's description of this organism

This is a new group not hitherto described The strains composing it give very regular biochemical reactions and have on the whole very distinctive appearances on blood agar Serologically they are quite distinct from the other groups, and there can be little doubt that they do form a distinct group of haemolytic streptococci The colonies tend to be somewhat smaller than those of group A (0 7–0 9 mm as against 0 8–1 3 mm), but they are usually of matt surface, and at first of greenish colour although tending to blacken after continued incubation The colonies themselves are hard, almost gritty in consistency and adhere closely to the medium so that they are difficult to dislodge The area of haemolysis produced is usually much the same as that of group A strains, but in the 8 per cent horse blood agar generally employed it is seldom quite complete, in Brown's 1 5 per cent agar with

5 per cent of horse blood it is, however, quite complete With continued incubation (48 hours or more) the haemolysis, particularly on 8 per cent agar, becomes alpha-prime in character, i e the area in the immediate neighbourhood of the colony becomes green and is surrounded by a zone of clear haemolysis Only one strain has failed to give this appearance On boiled blood agar, such strains give definite areas of green methaemoglobin, in which respect they differ from the strains of groups E and F and the majority of group K

Biochemically these strains are unable to form soluble haemolysin, they give a pH in 1 per cent glucose broth very slightly below that of group A strains, they have no action on hippurate or fibrin and they have the same fermentation reactions as group A strains

It is probable that they are the same as the strains isolated from the sputum of tuberculous patients by Cumming (1927) and named by him "pseudohaemolytic streptococci" because they resembled closely strains from infections but differed in being unable to form soluble haemolysin I have been unable to confirm his statement that such strains invariably lose their ability to give haemolysis on blood agar after more than four subcultures, although several strains have shown a tendency to throw off non-haemolytic colonies It is improbable that strains belonging to this group ever cause serious human infections, but seeing that they are relatively common in throat swabs and resemble group A strains superficially, it would seem that they have frequently been looked upon by previous workers as identical with pathogenic strains

The more extended description of the group H streptococcus contained in tables 1 and 2, covering characteristics not determined by Hare, is based upon the examination of only two of Dr Hare's cultures which were obtained for this study from Dr Lancefield If, however, these two cultures are representative of the group as a whole, it would appear from such characteristics as its ability to grow at 45°C, its thermal resistance, and its ability to ferment raffinose that this streptococcus differs radically from *Streptococcus pyogenes*

The Group K of Hare

Hare (1935) obtained eight cultures of streptococci from the human nose and throat which appeared to form a homogeneous and distinct serological group by the Lancefield precipitin

method, and for which he has tentatively suggested designation as group K As only these eight cultures of group K have been reported, and especially since Hare has suggested this group only provisionally, it has not been included in the tables of this review as representing a more or less established "species" Also, from Hare's description (and from the study of a few of Dr Hare's cultures) some doubt is felt as to whether most bacteriologists would classify these organisms as truly hemolytic streptococci Aside from his tabular material, Hare makes the following brief statement concerning this group

> This is a new and provisional group of which only eight representatives have been isolated The colonies tend to be about the same size as those of group A but they are moist and transparent, with crenated edges The area of haemolysis produced in 8 per cent horse blood agar is usually small and incomplete, being seldom more than 3 mm in diameter, although it may be larger and quite complete in Brown's 5 per cent agar An alpha-prime appearance is not produced
>
> Biochemically, these strains resemble group H strains in their inability to form soluble haemolysin, to dissolve fibrin or to act on hippurate Their pH in 1 per cent glucose broth is, however, higher, being 5 1 to 5 4, and seven out of the eight strains were unable to ferment trehalose There is thus some justification for looking on these strains as a separate group I have never encountered strains of this group in disease processes nor in other situations than the human throat

With the five fermentation test substances used, Hare found that lactose was fermented, and mannitol and sorbitol were not fermented While fermenting and non-fermenting strains were found with trehalose and salicin, six of the eight cultures fermented salicin but only one attacked trehalose They did not grow on blood agar containing 40 per cent bile

THE VIRIDANS STREPTOCOCCI

The "viridans" group, as here defined, is probably the least satisfactory of the divisions of the streptococci, and in all probability it is by far the most incomplete from the standpoint of the number of known types which may now be assigned to it The fairly clearly defined types which are included in this division

probably represent only a fraction of the non-hemolytic streptococci (alpha and gamma types) which fall outside of the lactic and enterococcus groups. Although the types recognized at present have a number of basic characteristics in common, it is highly improbable that all existing related non-hemolytic streptococci will be found to fit into this physiological pattern. The "viridans division" is therefore to be considered as probably having only a temporary usefulness in the present state of our ignorance of the streptococci belonging to this general group.

A great variety of human ailments have been ascribed to "*Streptococcus viridans*" and to "indifferent streptococci," and many specific names have been applied to organisms which were thought to have etiological importance in connection with those diseases. Although these inadequately described organisms are generally considered to be related to *Streptococcus salivarius*, how many of them belong to the established species of the non-hemolytic streptococci cannot be stated at the present time, and until some one takes the trouble to study them carefully we need not try to speculate upon their taxonomic relationships. In this connection, however, it may be pertinent to mention that according to Hucker (1937b), whose results have not yet been published, practically all of the strains contained in a collection of nearly 200 cultures of "*Streptococcus viridans*" obtained from clinical sources were found upon detailed physiological study to be either *Streptococcus salivarius* or *Streptococcus fecalis*, the larger portion belonging to the former species.

Serological methods have not proved useful tools for the classification of these streptococci. The specific carbohydrate appears to be type specific in the viridans streptococci (Lancefield, 1925a, 1925b, Hitchcock, 1924b), instead of group or species specific as in the case of the hemolytic forms. Hence they form a serologically heterogeneous group which *is not amenable*, for purposes of taxonomy, to the invaluable Lancefield technique. This is also in line with the experience gained with other serological methods, from the beginning of such studies the diversity of the non-hemolytic streptococci has usually been emphasized as compared with the relative homogeneity of the hemolytic

types (Kligler, 1915, Krumwiede and Valentine, 1916, Kinsella and Swift, 1917, 1918, Howell, 1918, Havens, 1919, Dochez, Avery and Lancefield, 1919, Clawson, 1920, Herrold, 1922, Norton, 1923, Crow, 1933) For example, Gordon (1922) found that of 131 cultures of *Streptococcus pyogenes*, 125 were of the same serological type as revealed by agglutination and agglutinin absorption tests, whereas with 16 cultures of *Streptococcus salivarius*, at least 12 different types were found by the same methods

In this division of the streptococci, therefore, the classification must at present rest entirely on physiological characteristics

TABLE 3

The viridans streptococci additional characteristics

SPECIES OR VARIETY	GROWTH IN 2 PER CENT NaCl	STARCH HYDROLYZED	SODIUM HIPPURATE HYDROLYZED	ESCULIN SPLIT	GELATIN LIQUEFIED	MILK CURDLED	FINAL pH IN GLUCOSE BROTH	ACID PRODUCED FROM: Arabinose	Maltose	Sucrose	Lactose	Trehalose	Raffinose	Inulin	Glycerol	Mannitol	Sorbitol	Salicin
S salivarius	+	−	−	±	−	±	5 4–4 0	−	+	+	+	±	±	±	−	−	−	±
S equinus	+	±	−	+	−	−	4 5–4 0	−	+	±	−	±	±	±	−	−	−	±
S bovis	+	+	−	+	−	±	4 5–4 0	±	+	+	+	±	+	±	−	∓	±	+
Varieties of *S bovis*	+	−	−*	+	−	±	4 5–4 0	−*	+	+	+	±	+	±	−	±	−	+*
S thermophilus	−	−	−*	−	−	+	4 5–4 0	−*	−*	+	+	−	±	−	−	−	−	−

* See table 1

Although these appear to be fairly satisfactory for the differentiation of at least a few types among the viridans streptococci, one need only reflect upon the revisions made necessary in our ideas about the groups of hemolytic streptococci with the advent of the Lancefield grouping, to realize what may happen at any time to the most carefully constructed creations of present day pattern builders

As has been previously pointed out and as is shown in table 1, the recognized types among the viridans streptococci show certain characteristics in common which justify their inclusion in

a separate division or group of the streptococci The combination of characteristics which appear to justify such a grouping is the inability to cause true beta hemolysis of blood, the possession of high minimum and relatively high maximum temperatures of growth, weak reducing action, a limited tolerance to methylene blue, salt and alkali, and the inability to produce ammonia from peptone In table 3 are given the additional characteristics of the members of this group

Streptococcus salivarius

Andrewes and Horder (1906) gave the name of *Streptococcus salivarius* to the organism which they found to be the predominating streptococcus of the human throat This organism was also sometimes found, probably only surviving rather than growing, in feces, and it was encountered in certain infections, especially endocarditis Streptococci of this general type had of course been studied before the work of Andrewes and Horder, there can be little doubt, for example, that some of the organisms studied by Schottmuller (1903) under the name of *Streptococcus mitior* were the same as *Streptococcus salivarius*, but since Andrewes and Horder were the first to describe this organism with sufficient detail to make its identity reasonably clear, the name *Streptococcus salivarius* appears to be the one which should be given priority for organisms of this general type

The outstanding characteristics which Andrewes and Horder ascribed to *Streptococcus salivarius* may be briefly stated as follows Blood is not hemolyzed, milk is acidulated and curdled, neutral red is usually reduced, lactose and sucrose are fermented, raffinose is usually fermented, but inulin less frequently, the glucosides, salicin and coniferin, may or may not be fermented, but mannitol is never fermented During a decade or more following the work of Andrewes and Horder, the tendency among bacteriologists was to use fewer tests in the characterization of streptococci, such characteristics as action on milk, reducing properties, and the fermentation of raffinose were largely dropped, and hence *Streptococcus salivarius* to some extent lost its identity in terms of its characterization by Andrewes and

Horder. Because of the various criteria which have been subsequently used by bacteriologists in the description of *Streptococcus salivarius*, it is pertinent to quote Andrewes and Horder (1906) on the subject: "It clots milk almost always and in its typical form reduces neutral red, though variants occur which fail to do this. The characteristic fermentation reactions are saccharose, lactose, and raffinose, the last named less constantly than the first two. The glucoside reactions may be added, and rarely inulin." Again they state: "The common positive chemical reactions are clotting of milk, reduction of neutral red, and acid formation with saccharose, lactose, and often raffinose, but not with mannite. Reactions with the glucosides are often added. . . ."

With the virtual discontinuance of some of the tests emphasized by Andrewes and Horder, *Streptococcus salivarius* has been described in various ways. In one system, this organism is defined as a non-hemolytic streptococcus which ferments lactose but does not ferment mannitol nor salicin. In another classification, which retains the raffinose reaction, *Streptococcus salivarius* is identified simply as non-hemolytic and fermenting raffinose but not mannitol.

Andrewes and Horder described as a separate species *Streptococcus mitis*, an organism found principally in the human mouth, which was very closely related to *Streptococcus salivarius* and which might, in fact, be considered simply as a variety of the latter species. *Streptococcus mitis* was described as not curdling milk, having only slight reducing action on neutral red, and usually not fermenting raffinose. Otherwise, its characteristics are the same as those of *Streptococcus salivarius*. Andrewes and Horder described *Streptococcus mitis* in these terms: "It is a short-chained form . . . and it gives a marked acid reaction in milk, though no clotting. . . . Its typical positive reactions on Gordon's tests are saccharose and lactose with or without the glucosides. . . . Such forms may with fair propriety be regarded as variants (of *Streptococcus salivarius*) in which the power of clotting milk has been suppressed."

It is obvious from the foregoing quotation that Andrewes and

Horder considered *Streptococcus mitis* as being so closely related to *Streptococcus salivarius* that its validity as a species was somewhat in doubt, and in recent years most bacteriologists have not attempted to differentiate such a species from *Streptococcus salivarius* However, in the useful and much-followed Holman (1916) classification, *Streptococcus mitis* is defined as being non-hemolytic, fermenting lactose and salicin, but not fermenting mannitol In this classification, *Streptococcus salivarius* and *Streptococcus mitis* have the same characteristics with the exception that *Streptococcus mitis* ferments salicin while *Streptococcus salivarius* does not

In a recent investigation of this group of streptococci Safford, Sherman and Hodge (1937) applied a broad list of physiological tests to the study of these organisms These investigators concluded that the type of streptococcus which occurs in largest numbers in the human throat is one which follows closely the description given by Andrewes and Horder for *Streptococcus salivarius* In blood agar the reaction varies from a complete gamma type in a few cultures to a marked alpha reaction in others, a weak alpha reaction being most characteristic What was considered the typical form of *Streptococcus salivarius* by these investigators is an active acid-producing organism which gives a final pH value in glucose broth of 4 4 to 4 0, and which acidulates and curdles milk promptly Although there is no reduction of litmus in milk cultures before curdling takes place, a marked and almost complete reduction occurs after coagulation A majority of cultures of this type ferment raffinose and also inulin, though the fermentation of inulin is less general

Although the description given in the above paragraph is considered accurate for *Streptococcus salivarius* in what Andrewes and Horder might call "its typical form," all the non-hemolytic streptococci from the human throat which belong to this same general group do not fall into such a rigid physiological pattern In a collection of such organisms there are also found many cultures which appear to agree in a general way with the type described by Andrewes and Horder as *Streptococcus mitis* No rigid line can be drawn between this somewhat indefinite variety

and the typical *Streptococcus salivarius* However, the mitis type is characterized by a weak acid-producing power, milk is not coagulated, and in glucose broth final pH values of 5 3 to 4 7 are attained These weak acid-producing forms usually do not ferment raffinose or inulin, and there also appears a tendency to produce a stronger or more definitely alpha type of action on blood than is true of the more typical form of *Streptococcus salivarius* Although there appear to be some more or less correlated divergencies from type, the differences between the so-called *Streptococcus mitis* and the characteristic form of *Streptococcus salivarius* are quantitative ones without any apparently rigid lines of demarcation Under these conditions, there does not appear to be a satisfactory basis for the establishment of *Streptococcus mitis* as a separate species at the present time However, it should be recognized that the more or less closely related non-hemolytic streptococci of the human throat undoubtedly form a rather complex group, which probably will be found to contain more than one species when more penetrating methods have been developed for the study of these organisms

Streptococcus equinus

Non-hemolytic streptococci which do not ferment lactose were first observed as contaminants from air Andrewes and Horder (1906) suspected that these organisms might come from horse dung, which at that time made up a large part of the organic pollution in the air of cities An investigation of fresh horse feces showed that this type of organism is the principal streptococcus found in the intestine of the horse, usually, in fact, constituting the majority of the total bacterial population of such material

This organism, which they named *Streptococcus equinus*, was described by Andrewes and Horder as being devoid of pathogenic properties, non-hemolytic, and having the following additional characteristics milk is not coagulated, there is little or no reducing action on neutral red, sucrose, salicin and coniferin are usually fermented, lactose and mannitol are not fermented, raffinose and inulin are not attacked as a rule, but a number of

variant types which ferment these substances were found Andrewes and Horder also pointed out the important fact that *Streptococcus equinus* has a high minimum temperature of growth, evidenced by little or no growth in gelatin cultures at 20°C They considered horse dung the chief source of the organism but thought that it might occur in the intestines of other herbivora They did not succeed in obtaining it from the intestines of certain carnivora examined

Winslow and Palmer (1910) likewise found *Streptococcus equinus* the predominating organism in the intestine of the horse and also reported the finding of similar non-lactose-fermenting streptococci in bovine and human feces Fuller and Armstrong (1913), Floyd and Wolbach (1914), Broadhurst (1915), Holman (1916), Blake (1917), Arnold (1920), and many others, have reported from various sources streptococci, which did not ferment lactose, under the name of *Streptococcus equinus* Aside from animal feces, such streptococci have been obtained frequently from the human mouth and also occasionally from the urine and from infections, though these organisms have not been clearly implicated as causative agents in such infections

Whether or not the streptococci failing to ferment lactose and which have been frequently obtained from human sources, especially the throat, are in fact the same as the true *Streptococcus equinus* of equine origin cannot be stated on the basis of present knowledge In recent years, the only requirements for an organism to be classified as *Streptococcus equinus*, or at least as a member of the "equinus group," are that it be a non-hemolytic streptococcus which fails to ferment lactose and mannitol Hodge and Sherman (1937) emphasized some of the little used tests of Andrewes and Horder, such as the minimum temperature of growth, and added a number of other characteristics in their description of this organism These investigators thought that the general "pattern" of reactions given by this organism, aside from its inability to ferment lactose, was sufficiently clear to mark it as a type

As may be seen from table 3, *Streptococcus equinus* shows points of close relationship to *Streptococcus salivarius*, on the

one hand, and to *Streptococcus bovis* on the other, in a number of respects it might be considered as falling between these two species Hodge and Sherman commented on the relationship of *Streptococcus equinus* to *Streptococcus bovis* and stated that although they suspected that *Streptococcus equinus* might hydrolyze starch, they found no evidence of this action when the cultures were tested in starch agar by the dilution, poured-plate method However, in more recent tests with a few typical cultures of *Streptococcus equinus* obtained from horse feces, it has been found that this organism may hydrolyze starch when a more favorable medium is used and the plates are inoculated by the streak method If the hydrolysis of starch should prove to be a general characteristic of the true *Streptococcus equinus*, this method might prove useful in the differentiation of this organism from aberrant strains of closely related types which fail to ferment lactose

In view of the unique characteristics of *Streptococcus equinus* and its close relationship to certain other species, a few comments are needed on its physiological characteristics which are not clear from the tabular material presented An outstanding feature is its high minimum temperature of growth of about 20°C *Streptococcus equinus* grows poorly in milk, even with added glucose, and it has very slight reducing action Although sucrose, raffinose, inulin and salicin may or may not be fermented, sucrose and salicin are fermented by a large majority of strains, whereas raffinose and inulin are attacked by a relatively small percentage of cultures

Compared with *Streptococcus salivarius*, *Streptococcus equinus* appears to have a somewhat higher maximum temperature of growth and a slightly higher thermal resistance, and less frequently ferments raffinose and inulin On the other hand, *Streptococcus bovis* has a higher thermal death point than *Streptococcus equinus*, more frequently ferments raffinose and inulin, and usually ferments arabinose

In the Holman (1916) classification, which is based on four characters, strains of the "equinus group" which do not ferment salicin are designated as *Streptococcus ignavus* In this system,

the difference between *Streptococcus equinus* and *Streptococcus ignavus* rests entirely on the salicin reaction, both species being otherwise described as non-hemolytic and failing to ferment lactose and mannitol

Streptococcus bovis

Winslow and Palmer (1910) and Fuller and Armstrong (1913) observed that the prevailing type of streptococcus in the feces of the cow ferments raffinose, while the predominating forms from the intestines of man and the horse do not have this property As their studies were largely limited to the fermentation tests, Fuller and Armstrong considered this organism, on the basis of the raffinose reaction, as identical with the *Streptococcus salivarius* of the human throat

Streptococcus bovis was described as a new species and named by Orla-Jensen (1919), who obtained it from cow feces and from milks which had been heated or incubated at high temperatures Although obtained from a number of sources, this streptococcus was recognized as having its habitat in the bovine alimentary tract Although Orla-Jensen did not study *Streptococcus salivarius*, his description of *Streptococcus bovis* is nevertheless adequate to indicate strongly, if not thoroughly to establish, its integrity as an independent species Aside from being somewhat unusual with respect to the fermentation tests, *Streptococcus bovis* was observed to have rather marked thermal resistance and to grow at relatively high temperatures With respect to substances which are not generally fermented by streptococci, *Streptococcus bovis* was found to be an active fermenter of arabinose, raffinose, starch, and usually inulin

Ayers and Mudge (1923) found *Streptococcus bovis* to be the predominating streptococcal form in the mouths and intestines of cows Aside from the fermentation tests, these investigators studied *Streptococcus bovis* along somewhat different lines and added further information concerning its characteristics, such as its inability to produce ammonia from peptone, its weak reducing action, its action on sodium hippurate, blood, etc Sherman and Stark (1931) also studied *Streptococcus bovis*, combining the methods of Orla-Jensen and of Ayers and Mudge,

and confirmed the results of these investigators Sherman and Stark also paid especial attention to the temperature limits of growth, and noted as a particular characteristic of *Streptococcus bovis* its ability to hydrolyze starch actively when tested by the starch agar method

Although the relationship between *Streptococcus bovis* and *Streptococcus salivarius* is obviously close, an inspection of the data given in tables 1 and 3 indicates a number of divergencies between these two types Compared with *Streptococcus salivarius*, *Streptococcus bovis* has a somewhat higher maximum temperature of growth, a distinctly greater thermal resistance, hydrolyzes starch, and usually ferments arabinose, some strains ferment mannitol Those who have worked intimately with these two groups of organisms feel that they are distinct types, but it must be admitted that convenient and decisive tests for their differentiation are still needed The confusion between *Streptococcus bovis* and *Streptococcus salivarius* becomes greater in the case of the non-starch-hydrolyzing varieties of the "bovis group"

Streptococcus bovis has been isolated from human feces, and what appear to be simply varieties of this organism, which do not hydrolyze starch, are commonly found in the human intestine The question as to whether or not *Streptococcus bovis*, as in the case of a number of other non-pathogenic streptococci, may occasionally be found in human infections, cannot be answered as the methods ordinarily used in the study of non-hemolytic streptococci from clinical sources are not adequate for the identification of this organism As will be noted later, some strains of the so-called "Bargen streptococcus," thought by some to be associated with ulcerative colitis, have proved to be starch-hydrolyzing streptococci which appear to be identical with *Streptococcus bovis*

Varieties of Streptococcus bovis Streptococcus inulinaceus and the "Bargen Streptococcus"

Streptococcus inulinaceus was so named by Orla-Jensen (1919) who described this type as an independent species, though noting that it was very closely related to *Streptococcus bovis* and

might be considered as a variety of that species The type described as *Streptococcus inulinaceus* does not ferment starch, or ferments it only weakly, and does not ferment arabinose, substances which are actively fermented by the typical *Streptococcus bovis* Sherman and Stark (1931) also found that the members of the "bovis group" which did not hydrolyze starch also failed to ferment arabinose, while the fermentation of arabinose appeared to be perfectly correlated with the hydrolysis of starch in their strains of the typical *Streptococcus bovis*, their results therefore tended to add some validity to *Streptococcus inulinaceus* as an independent type, though it was suggested that it probably should be considered as a variety of *Streptococcus bovis*

In a recent study of the *Streptococcus bovis* group (Stark and Sherman, 1937) even less grounds have been found for considering *Streptococcus inulinaceus* as a separate species A considerable proportion of starch-hydrolyzing strains have been obtained which do not ferment arabinose, thus spoiling the correlation which was indicated by previous work Earlier work had also indicated that the so-called *Streptococcus inulinaceus* is especially characteristic of the bovine throat, while *Streptococcus bovis* is the predominating type in the intestine, but this has not been confirmed by more recent findings, the typical starch-hydrolyzing *Streptococcus bovis* being the prevailing form in both the mouth and the feces Unless very fine lines are to be drawn in the establishment of species in this group, there would not appear to be sufficiently substantial grounds for considering the non-starch-hydrolyzing type as more than a variety of *Streptococcus bovis*

What has become colloquially known as the "Bargen streptococcus" is a form which Bargen (1924, 1930) considers as having a causative relationship to ulcerative colitis For our present purposes it is not pertinent to review the considerable body of literature, chiefly clinical, which has grown up around this moot question However, reference may be made to the recent papers of Torrey and Montu (1934, 1936) which contain reports on studies of the organism, as well as citations to the literature The identity of the "Bargen streptococcus" has remained ob-

scure Some have identified the organism simply as a somewhat heat-tolerant diplococcus or short-chained streptococcus Others have specified that it must be able to ferment raffinose but not mannitol Torrey and Montu (1936) showed, contrary to one claim which had been made, that the "Bargen streptococcus" is not identical with or closely related to *Streptococcus mastitidis*, on the other hand, they found it to have some properties in common with the enterococci and concluded that it is a variant of the enterococcus

Through the kindness of Dr Luther Thompson, who furnished ten cultures of the "Bargen streptococcus" isolated from cases of ulcerative colitis, it has been possible to study this organism in comparison with the well established types of streptococci (Stark and Sherman, 1937) These cultures were found to have very little indeed in common with the enterococci but to be, on the other hand, very closely related to, if not identical with, *Streptococcus bovis* As previously indicated, five of these cultures had the ability to hydrolyze starch, and, on the basis of present methods of studying streptococci, would have to be considered identical with the typical form of *Streptococcus bovis*, the other five cultures were not able to hydrolyze starch but otherwise agreed entirely with the variants of this species which do not attack starch With respect to the non-starch-hydrolyzing variety, it may be of some interest to note that a small collection of this type has been isolated from the feces of a number of normal people, thus indicating that what appears to be the typical "Bargen streptococcus" is commonly found in the human intestine

Streptococcus thermophilus

Streptococcus thermophilus was discovered by Orla-Jensen (1919) who gave an excellent description of this unique organism The typical *Streptococcus thermophilus* is a very distinct type with physiological characteristics which differentiate it clearly from any other known streptococcus It is here included among the "viridans" streptococci since in its basic characteristics it is most closely related to that group, but the properties of the or-

ganism are so distinctive that it could well be considered as belonging to an independent division of the streptococci

Although no known streptococcus is truly thermophilic, *Streptococcus thermophilus* grows very actively at 50°C and slightly above, but growth does not take place at 53°C It also has a high thermal death point—apparently slightly higher than that of the other more heat-resistant streptococci—which allows it to survive, in large numbers, heat treatments of 60 to 65°C, such as in the pasteurization of milk On the other hand, it has a high minimum temperature of growth—about 20°C —and is extremely fastidious in its nutritive requirements It usually has no detectable action on red blood cells, being completely "indifferent" in blood agar cultures *Streptococcus thermophilus* does not grow in simple solid or liquid media containing only beef extract and peptone, but demands a richer medium or one supplemented with appropriate carbohydrate material Even on the most favorable solid medium, *Streptococcus thermophilus* produces only very small "pin-point" colonies, and a general characteristic of the organism is its lack of viability in artificial media, causing it to be easily lost in laboratory cultures

As may be noted from table 3, *Streptococcus thermophilus* is marked more by the things which it cannot do than it is by its positive reactions It is one streptococcus which could be almost positively identified on the basis of its fermentation reactions alone Its almost invariable inability to ferment maltose is unique In our own work covering many cultures, in addition to those which have been published (Sherman and Stark, 1931), we have not had a strain of this organism which fermented maltose, but Orla-Jensen (1919) and others have noted a slight fermentation of this sugar by some strains *Streptococcus thermophilus* seldom ferments raffinose and has never been reported to ferment inulin, glycerol, mannitol, sorbitol or salicin Orla-Jensen (1919) noted that *Streptococcus thermophilus* is especially favored by sucrose and produces a strong fermentation of this substance, at the same time he noted that the monosaccharide mannose was only weakly fermented Wright

(1936a, b) has confirmed and extended these observations He has shown that certain strains of *Streptococcus thermophilus* utilize lactose and sucrose more readily than the constituent monosaccharides, as indicated by a faster rate of fermentation On the basis of these results, Wright believes that *Streptococcus thermophilus* ferments the disaccharides lactose and sucrose without preliminary hydrolysis Among the other unusual characteristics of *Streptococcus thermophilus* is its extreme sensitiveness to sodium chloride, it being inhibited by only 2 per cent of this salt No other known streptococcus is so sensitive to salt, and in this respect, as well as in certain other characteristics, *Streptococcus thermophilus* shows a physiological kinship to *Lactobacillus bulgaricus*

Streptococcus thermophilus is in general characterized by the production of fairly long chains in liquid media, but in this respect it shows the variability which is characteristic of practically all streptococci According to Orla-Jensen, this organism forms longer chains at its optimum temperature of around 45°C than at lower temperatures, in this respect differing from the behavior of some streptococci

The habitat of *Streptococcus thermophilus* is not known It has never been obtained from clinical sources, and from its physiological nature it is doubtful if this organism could even rarely be implicated as a secondary invader of the tissues Although its temperature requirements for growth might suggest that it is an intestinal organism, there is no proof that this is its source *Streptococcus thermophilus* has been isolated only from milk and milk products It can sometimes be isolated from quantitative plates made directly from pasteurized milk It can also usually be isolated from milk which is incubated at 50°C and plated soon after curdling, before the lactobacilli have gained ascendancy *Streptococcus thermophilus* can frequently be isolated from Swiss cheese only a day or two old, since in the manufacture of this cheese a "cooking" temperature between 50 and 55°C is employed, and the freshly made curd cools slowly and remains above 40°C for about 15 hours, thus providing ideal temperature conditions for the growth of the organism In

this connection, *Streptococcus thermophilus* has acquired a technical importance in that it is now sometimes used as a "starter" for the inoculation of milk in the manufacture of Swiss cheese (Frazier, 1933, Frazier, Burkey, Matheson and Watson, 1933)

THE LACTIC STREPTOCOCCI

Since all streptococci produce lactic acid as the chief product of fermentation, it is misleading to designate any particular group as "lactic" organisms However, the ordinary milk-souring organism has so long been known as the "lactic-acid streptococcus" that the term has acquired a familiar technical meaning The lactic streptococci form a homogeneous and quite distinct group They are characterized by having low minimum and maximum temperatures of growth, strong reducing action, and a marked tolerance to methylene blue (Sherman and Albus, 1918) These features, supplemented by the other physiological characteristics of the organisms, mark rather clearly the boundaries of the group

From the pyogenic and the viridans divisions of the streptococci, the lactic organisms are markedly differentiated by their ability to grow at 10°C , their strong reducing action, and their ability to grow in the presence of a relatively concentrated (0 1 per cent) solution of methylene blue in milk cultures From the enterococci, on the other hand, the lactic streptococci are differentiated by their inability to grow at 45°C , their inability to grow in the presence of 6 5 per cent sodium chloride, the inhibition of their growth in an alkaline medium with a pH of 9 6, and a somewhat lower thermal resistance (Sherman and Stark, 1934, Sherman, Stark and Yawger, 1937)

The lactic streptococci have acquired commercial importance through their use as "starters" in the dairy industry They are used for the "ripening" of cream for buttermaking, for the inoculation of milk for cheesemaking, in order to produce the initial lactic acid fermentation in the curd, and for the production of artificial buttermilk and certain other types of fermented milk drinks These useful functions, together with the rôle they play in the natural souring of milk with the suppression of putrefactive and other obnoxious bacteria, have caused

the lactic streptococci to be looked upon as desirable organisms and friends rather than foes of mankind

Although some diversity among different types of lactic streptococci is indicated on the basis of fermentation reactions and certain other characteristics, most workers have considered these only variants from type, or at most varieties, rather than species (Ayers, Johnson and Mudge, 1924, Hammer and Baker 1926, Stark and Sherman, 1935, Yawger and Sherman, 1937a) On the other hand, there have been suggestions, notably by Orla-Jensen and Hansen (1932), that this group of streptococci should be divided into a number of species based upon fermentation

TABLE 4

The lactic streptococci additional characteristics

SPECIES	AMMONIA PRODUCED FROM PEPTONE	GROWTH AT 40°C	GROWTH IN PRESENCE OF: 4 per cent NaCl	pH 9.2	0.3 per cent methylene blue in milk	SODIUM HIPPURATE HYDROLYZED	STARCH HYDROLYZED	ESCULIN SPLIT	GELATIN LIQUEFIED	MILK CURDLED	FINAL pH IN GLUCOSE BROTH	ACID PRODUCED FROM: Arabinose	Xylose	Maltose	Sucrose	Lactose	Raffinose	Inulin	Glycerol	Mannitol	Sorbitol	Salicin
S lactis	+	+	+	+	+	±	−	+	−	+	4.5–4.0	±	±	+	±	+	−*	−	−	±	−	±
S cremoris	−	−	−	−	±	−	−	±	−	+	4.6–4.0	−	−	±	±	+	−*	−	−	−*	−	±

* See table 1

reactions Ignoring such minor distinctions, there nevertheless appear to be two fairly clearly defined species in the group, *Streptococcus lactis* and *Streptococcus cremoris* The physiological characteristics of these organisms, other than those given in table 1, are included in table 4

The data given in table 4 bring out rather clearly the physiological differences between *Streptococcus lactis* and *Streptococcus cremoris*, other facts supporting this differentiation will be given in the discussion of the individual species

Streptococcus lactis

Streptococcus lactis was the first streptococcus described, being studied by Lister (1873, 1878) who isolated it from milk and

named it *Bacterium lactis* There is nothing surprising in the fact that Lister gave this organism the generic name *Bacterium*, it should be remembered that *Streptococcus* as a generic name had not yet come into use, and Lister observed differences between his organism and the commonly known micrococci of his time As the predominating organism in sour milk and as the chief causative factor in the souring process, *Streptococcus lactis* has been known through the years under a number of names, of which *Streptococcus acidi-lactici* (Grotenfeldt), *Bacterium lactis-acidi* (Leichmann) and *Streptococcus lacticus* (Kruse) had the widest usage Following the lead of Lohnis (1909) bacteriologists have almost universally adopted the specific name applied by Lister, so that the organism is now generally known as *Streptococcus lactis*

Although *Streptococcus lactis* has long been known and extensively studied, its exact identity was not clarified until comparatively recently On the other hand, because of its rapidity of growth in milk with its resulting tendency to predominate in the natural souring of milk, there can be little doubt that most of the older work done on this organism really dealt with the true *Streptococcus lactis* The complete reduction of litmus before curdling in milk cultures was looked upon as an especially characteristic property of the organism (Hastings, 1911), but this characteristic was even then known not to be peculiar to *Streptococcus lactis* Andrewes and Horder (1906) had shown the strong reducing action of *Streptococcus fecalis* on neutral red, and MacCallum and Hastings (1899) had shown the ability to reduce litmus in milk cultures before curdling to be an especially characteristic feature of the organism which now goes under the name of *Streptococcus zymogenes* Sherman and Albus (1918) showed that, in addition to other characteristics, *Streptococcus lactis* has a minimum growth temperature below 10°C and a maximum temperature for growth at about 43°C, and that these properties are correlated with the characteristic action in litmus milk The strong reducing action of the organism was also established with indigo carmine and neutral red, its tolerance to methylene blue was noted, as were also the fermentation reac-

tions Orla-Jensen (1919) emphasized especially the fermentation reactions and recorded the action of *Streptococcus lactis* on a number of additional substances Ayers, Johnson and Mudge (1924) considered the three most important differential characteristics of *Streptococcus lactis* to be its strong reducing action on litmus and Janus green, its ability to grow at 10°C, and its ability to produce a low final pH in media of low surface tension These investigators were perhaps the first to study thoroughly the action of *Streptococcus lactis* on blood They showed that the colonies of this organism on blood agar plates, while frequently of the gamma type, might also show various degrees of coloration through a weak alpha to a more typical alpha reaction, and these results have been amply confirmed by others

Streptococcus lactis is generally referred to as being characteristically a diplococcus rather than a typical chain-forming streptococcus, but this varies greatly with different strains and with the conditions of growth Some strains of this organism produce good chains in ordinary media Heinemann (1906) showed that *Streptococcus lactis* produces chains in liquid media containing blood serum, and Sherman and Albus (1918) found the same to be true in a bile medium

In fermentation reactions there is considerable diversity among different strains of *Streptococcus lactis,* among the substances which may or may not be fermented being arabinose, xylose, sucrose, mannitol and salicin Orla-Jensen and Hansen (1932) have reported a strain which ferments raffinose, and Yawger and Sherman (1937a) have encountered a few cultures which do not ferment lactose Starch is not hydrolyzed, when tested by the plate method, though a few strains have been noted which have a very slight action on this substance That at least a portion of the diversity observed in the fermentation tests with the lactic-acid streptococci is to be ascribed to variation, rather than indicating distinct varieties, appears to be established (Sherman and Hussong, 1937)

There should be little need at this late date to discuss the old question of the relationship of *Streptococcus lactis* to *Streptococcus*

fecalis, but since these organisms have been confused in recent papers (Kleckner, 1935, Chapman, 1936), the subject will be briefly discussed Until recent years there were no methods at hand for the differentiation of these two organisms, and in some of the older and better known classifications of the group *Streptococcus lactis* was not recognized as an independent species Ayers and Johnson (1924) showed that *Streptococcus lactis* and *Streptococcus fecalis* were similar in having strong reducing action, and low minimum temperatures of growth, and in being able to produce low final pH values in media of reduced surface tension, as well as showing considerable similarity in morphological and general cultural characteristics The work of these distinguished investigators naturally gave great weight to the view that *Streptococcus lactis* and *Streptococcus fecalis* were identical Since that time, however, methods have been applied to the study of the streptococci which appear to differentiate very clearly these two species (Sherman and Stark, 1931, 1934, Sherman, Mauer and Stark, 1937) As was shown in table 1, *Streptococcus fecalis* has a higher maximum temperature of growth and is more tolerant to salt and alkali than is *Streptococcus lactis* In addition, *Streptococcus fecalis* has a slightly higher thermal death point than does *Streptococcus lactis* Even on the fermentation tests the two species appear to be fairly distinct *Streptococcus lactis* does not ferment glycerol or sorbitol and may or may not ferment mannitol, *Streptococcus fecalis*, on the other hand, usually ferments both mannitol and sorbitol, and frequently ferments glycerol

The true *Streptococcus lactis* is not known to occur in natural infections of man or animals However, Heinemann (1907) reported the building up of virulence in cultures of *Streptococcus lactis* by passage through rabbits Hammer (1928), on the other hand, reports experiments in which the findings of Heinemann were not confirmed Various cultures of *Streptococcus lactis* isolated from milk did not prove to be harmful to rabbits or guinea pigs When injected intravenously into rabbits, the organisms could be recovered from the spleen and liver for a number of hours after inoculation, but repeated passage through rabbits failed to yield cultures capable of causing death

The habitat of *Streptococcus lactis* has been somewhat of a mystery It has long been established that this organism is not a normal inhabitant of the bovine udder (Rogers and Dahlberg, 1914, Evans, 1916, Ayers and Mudge, 1922), and although older reports had indicated that the source of *Streptococcus lactis* might be the mouths and intestines of cows, Ayers and Mudge (1923), with more modern methods of study, did not find this organism among the characteristic streptococci of the bovine mouth, throat, or feces Stark and Sherman (1935) isolated *Streptococcus lactis* repeatedly from certain plants, but not from all plants examined On the basis of these findings it was suggested that plants may represent the natural habitat of *Streptococcus lactis*, though definite conclusions to this effect were not drawn In view of the fact that *Streptococcus lactis* does commonly occur on plant materials, it would seem likely that surviving strains would sometimes be found in the feces of animals

Streptococcus cremoris

There has long been a belief among some of those engaged in the scientific aspects of the dairy industry that the best lactic-acid streptococci for "starter" use have more tendency to form chains and to produce a slightly viscous body in milk cultures than does the typical *Streptococcus lactis* obtained from spontaneously soured milk Such an ill-defined type has for years been known as a variety of *Streptococcus lactis* and also under the specific name of *Streptococcus hollandicus* (Weigmann) This organism was given more standing as an independent type by the work of Orla-Jensen (1919) who described it as a new species under the name of *Streptococcus cremoris* He showed that *Streptococcus cremoris* is usually a more typical chain-forming streptococcus than is *Streptococcus lactis*, that it frequently fails to grow at 37°C, usually produces less acid in milk, and in general has less fermentative power than does *Streptococcus lactis*, especially on maltose and dextrin Although the work of Orla-Jensen indicates the validity of *Streptococcus cremoris* as a separate species, the differences between this organism and *Streptococcus lactis*, as defined by him, are relative or quantitative ones rather than definitive

Quite independent of the work of Orla-Jensen, and uncorrelated with it, is that of Ayers, Johnson and Mudge (1924) who described a unique type of lactic-acid streptococcus as *Streptococcus lactis* Var B The outstanding feature of this "B" variety was its inability to produce ammonia from peptone, while the typical *Streptococcus lactis* is able to produce this substance in 4 per cent peptone solutions Other less sharply defined characteristics of *Streptococcus lactis* Var B were a higher average limiting pH in glucose broth and a less vigorous action in milk cultures than that of the typical *Streptococcus lactis*

Suspecting that the organisms described by Orla-Jensen and by Ayers, Johnson and Mudge were the same, Yawger and Sherman (1937b) followed the lead of the latter and from commercial starters and milk isolated cultures of lactic streptococci which did not have the ability to produce ammonia from peptone By the application of some new tests, it was found possible to correlate with the inability to produce ammonia other characteristics which differentiated this type from the typical *Streptococcus lactis* This type in turn also had the characteristics of Orla-Jensen's *Streptococcus cremoris* As may be seen from table 4, *Streptococcus cremoris* is not only unable to produce ammonia from peptone, but is also unable to grow at 40°C, in the presence of 4 per cent sodium chloride, or in an alkaline medium of pH 9 2, *Streptococcus lactis*, on the other hand, is able to produce ammonia from peptone, and is *not* inhibited in its growth by a temperature of 40°C, by 4 per cent sodium chloride, nor at a pH of 9 2 *Streptococcus lactis* also shows a greater average tolerance to methylene blue and in general has greater fermentative power than does *Streptococcus cremoris*, but a clear differentiation apparently cannot be based on the fermentation tests

With regard to morphology, *Streptococcus cremoris* is, as a rule, more typically chain-forming than is *Streptococcus lactis*, however, some strains occur more typically as diplococci, while some strains of *Streptococcus lactis* produce chains, thus preventing a clear-cut distinction on this basis In many cases the cells of

Streptococcus cremoris are distinctly larger than those of *Streptococcus lactis*, but again there are so many exceptions to the rule that this characteristic cannot be relied upon It may be said, however, that lactic streptococci which are characterized both by large cells and by the formation of long chains in milk cultures offer presumptive evidence of being *Streptococcus cremoris* rather than *Streptococcus lactis*

Generally speaking, *Streptococcus cremoris* does not grow so well in artificial media as does *Streptococcus lactis*, and it is also slightly less acid-tolerant, as revealed by a lower average production of acidity in milk and by usually not reaching quite so low final pH values in glucose broth

Streptococcus cremoris has been isolated only from milk and milk products As this organism not only cannot grow at 40°C but a majority of strains do not grow even at 37°C, it seems unlikely that *Streptococcus cremoris* occurs as an animal parasite and scarcely possible that it could ever be implicated as an agent of disease

THE ENTEROCOCCI

Since its use by Thiercelin (1899a, 1899b, 1902) the term "enterococcus" has had a somewhat variable and hazy meaning In some cases the name has been applied rather specifically as a synonym for *Streptococcus fecalis*, but most workers have used "enterococcus" in a loose group sense to designate the fecal streptococci which have in common some of the outstanding characteristics of *Streptococcus fecalis* A fecal diplococcus or short-chained streptococcus somewhat resistant to heat, with the ability to ferment mannitol and a tolerance for bile, would fully meet the requirements of most investigators for classification as an enterococcus, while some workers have so classified their organisms without the use of all of these characteristics It should be noted that although the foregoing features are characteristic of the enterococci, not one of these properties is limited to that group of the streptococci Through the application of a wider assortment of tests, and from more extensive

studies of the individual species, there has gradually evolved a rather definite and clearly defined enterococcus division of the streptococci

Although there is a large body of literature dealing with the enterococci, only a few of these papers record the characteristics of the organisms dealt with in sufficient detail to make it profitable to review them The older literature was reviewed by Dible (1921) and the more recent literature has been reviewed by a number of workers (Bagger, 1925, 1926, Dible, 1929, Demeter, 1929) Dible's contribution was an excellent one, and among the other worthwhile contributions should be mentioned the work of Bagger (1926) who used a wide assortment of test substances and also made significant observations on the temperature and pH range of growth of the enterococci In general, however, it may be fairly said that most of the more significant contributions to our knowledge of the nature of the enterococci have been made by those workers who have studied individual species, rather than dealing with the "enterococcus" in the loose and hazy sense in which this term has more generally been used

TABLE 5

The enterococci additional characteristics

SPECIES	LANCEFIELD GROUP	HEMOLYSIS	GELATIN LIQUEFACTION	STRONG REDUCTION	ACTIVELY FIBRINOLYTIC	SODIUM HIPPURATE HYDROLYZED	STARCH HYDROLYZED	ESCULIN SPLIT	MILK CURDLED	FINAL pH IN GLUCOSE BROTH	ACID PRODUCED FROM Arabinose	Maltose	Sucrose	Lactose	Trehalose	Raffinose	Inulin	Glycerol	Mannitol	Sorbitol	Salicin
S fecalis		−	−	+*	−	±	−	+	+	4 5-4 0	±	+	±	+	+	±	−*	±	+*	+	+
S liquefaciens		−	+	+*	−	±	−	+	+	4 5-4 0	±	+	+*	+	+	±	−	+	+	+	+
S zymogenes	D	+	±	+*	−	±	−	+	+	4 5-4 0	±	+	+*	+	+	±	−	+*	+	+	+
S durans		+	−	−	−	±	−	+	+	4 5-4 0	−	+	−*	+	±	−	−	−	−*	−	±

* See table 1

On the other hand, it should be admitted that some of the

"species" of the enterococcus division of the streptococci are separated from each other by rather thin and shaky boundaries, as may be seen from an inspection of table 5

The enterococci present many points of interest Not only are they characterized by unique physiological properties, as was shown in table 1, but in this group of streptococci there is a merging of hemolytic and non-hemolytic strains in what appears to be an otherwise physiologically homogeneous type, and in this group, which contains the only known proteolytic streptococci, there is likewise a fusion of proteolytic and non-proteolytic strains in the same otherwise homogeneous types, for which only convenience justifies designation as separate species As was previously mentioned, motility in the streptococci has been especially allied with members of the enterococcus group

Although the enterococci are considered as having their origin in the intestines of man and other warm-blooded animals, the resistance and tolerance of these streptococci, together with their low minimum and high maximum temperature limits of growth, not only fit them to survive but also to grow under diverse conditions in nature The so-called *Streptococcus apis* (Maassen) which is associated, probably only as a secondary invader, with European "foulbrood" of bees appears to be an enterococcus Thompson and Thompson (1928) claimed the identity of *Streptococcus apis*, *Streptococcus zymogenes* and *Streptococcus liquefaciens*, Hucker (1932) found the cultures of *Streptococcus apis* studied by him to be similar to *Streptococcus liquefaciens*, while Davis and Tarr (1936), who studied both proteolytic and non-proteolytic types, identified them with *Streptococcus liquefaciens* and *Streptococcus fecalis* ("*Streptococcus glycerinaceus*") The enterococci grow well in milk and certain milk products, notably cheese This fact has been partially responsible for the widespread confusion of *Streptococcus fecalis* and *Streptococcus lactis*, while certain of the hemolytic members of the enterococcus group have sometimes been designated as "dairy types" Unpublished investigations have shown enterococci of the *Streptococcus fecalis* and *Streptococcus liquefaciens* types to occur rather commonly on

plants This may of course mean that these organisms were merely surviving, rather than growing, under these conditions An interesting point in this connection, and one which might be considered as evidence against such an accidental occurrence is the fact that none of the hemolytic types of the enterococci have thus far been isolated from plant materials, but no systematic investigation has been made with the specific object of obtaining these types of hemolytic streptococci from vegetable sources

Streptococcus fecalis

Streptococcus fecalis was named by Andrewes and Horder (1906) who gave an excellent description of the organism in view of the means then at hand for studying bacteria The organism was described as non-hemolytic, having strong reducing action on neutral red, coagulating milk and fermenting the disaccharides, salicin, and mannitol, but not inulin and usually not raffinose The fermentation of mannitol was considered very characteristic *Streptococcus fecalis* was found to be the predominating streptococcus in human feces and from that time it has been known as the most characteristic streptococcal type of the human intestine

The work of Andrewes and Horder was verified by a number of subsequent workers on intestinal streptococci (Winslow and Palmer, 1910, Fuller and Armstrong, 1913, Broadhurst, 1915, and others) In later classifications the identity of *Streptococcus fecalis* became less distinct, as bacteriologists came to depend more on a smaller number of tests and attention to some of the important physiological reactions was largely discontinued In one such classification (Gordon, 1922) *Streptococcus fecalis*, or the "*enterococcus*," was identified merely as a non-hemolytic streptococcus which fermented mannitol but not raffinose

Dible (1921), however, subjected the intestinal streptococci to a careful study which resulted in a broader and more accurate description of *Streptococcus fecalis* Ayers and Johnson (1924) likewise studied this organism on a broader base, taking into consideration a variety of physiological characteristics Although

he did not integrate his work with that of other investigators, and used new terminology, Orla-Jensen (1919) nevertheless made valuable additions to the existing knowledge of the physiology of *Streptococcus fecalis*, which he described under the specific names of *Streptococcus faecium* and *Streptococcus glycerinaceus*. Although Orla-Jensen based his classification mainly on the results of fermentation tests with 18 substances, he made valuable observations on the thermal resistance and the temperature ranges of growth of the organisms studied. As previously noted, especial emphasis has been laid in recent studies on the growth tolerance of *Streptococcus fecalis* and other enterococci to relatively high and low temperatures and to a number of substances which are in general inhibitory to other streptococci.

Assuming, in deference to custom and convenience, that the closely related hemolytic and proteolytic types should be considered as distinct species, the remaining organisms which are generally classified as *Streptococcus fecalis* form a rather homogeneous physiological group. There appears to be relatively little variation from type so far as the more basic characteristics are concerned, but it is likely that deviation from the usual pattern will be found more common as experience increases and more incisive tests are developed and applied to the study of non-hemolytic streptococci. For example, strong reducing action, long known as an outstanding characteristic of *Streptococcus fecalis*, is found to be lacking in a small proportion of cultures (Sherman, Mauer and Stark, 1937) and a few strains appear to lose this property on long cultivation in the laboratory.

With respect to the fermentation tests, however, *Streptococcus fecalis* shows great diversity, a fact which has been noted by all workers who have applied a broad list of test substances to the study of this organism. Arabinose, sucrose, raffinose and glycerol may or may not be fermented. Although the fermentation of mannitol has long been looked upon as an especially constant characteristic of *Streptococcus fecalis*, a few strains fail to attack this substance, as was shown by Dible (1921), whose observation has been confirmed by others. Inulin is only rarely attacked, but a small percentage of strains are able to ferment it.

Although suggestions have been made that the group should be subdivided on the basis of the fermentation reactions—for example, the *Streptococcus glycerinaceus* of Orla-Jensen—Sherman, Mauer and Stark (1937) were unable to find correlations which appeared to justify such a procedure

In addition to being the most abundant streptococcus in the human intestine, it is probable that *Streptococcus fecalis* occurs generally in other animals, most positively in certain ones The fact that some animals, notably the horse and the cow, harbor other types which make up the prevailing streptococcus flora has led to statements which imply that *Streptococcus fecalis* is peculiar to the human intestinal tract, but a number of investigators have reported this organism as occurring in the feces of horses, cattle and other domestic animals

Although *Streptococcus fecalis* is not to be classed as a pathogenic organism—scarcely more so, one would think, than *Bacterium coli*—it has been known since the time of Andrewes and Horder (1906) as occasionally occurring in cases of endocarditis and other human infections Houston (1936) has noted many types of infections in which enterococci appeared to be implicated It is still an open question how frequently the "*Streptococcus viridans*" of clinical workers is in fact *Streptococcus fecalis*

Streptococcus liquefaciens

The occurrence of streptococci which liquefy gelatin was known in the early days of bacteriology, and the older literature records the names of many streptococci which were supposed to have this property One of the first such types described and the one which is frequently given priority is the *Streptococcus coli-gracilis* (Escherich) However, it is impossible now to identify any of these organisms from their recorded descriptions

The name *Streptococcus liquefaciens* was first used by Sternberg (1892) for a streptococcus which, it must be admitted, could not now be recognized from his description Orla-Jensen (1919) revived the name and applied it to a group of proteolytic streptococci which he studied It is of interest to note that Orla-Jensen established this species around a type culture which von

Freudenreich (1894) had described many years before under the name of *Micrococcus casei-amari* *Streptococcus liquefaciens* has been studied by a number of investigators since Orla-Jensen, and reference may be made to the recent work of Long and Hammer (1936) in which the literature is reviewed

Some rather fine lines have been drawn in the establishment of species in the enterococcus group and the standing of *Streptococcus liquefaciens* in this respect may well be questioned As is shown in table 5, the only clear-cut difference between this so-called species and *Streptococcus fecalis* is proteolytic action, while from proteolytic strains of *Streptococcus zymogenes*, on the other hand, the differentiation is based solely on hemolytic activity There is, therefore, only a slender basis for considering *Streptococcus liquefaciens* as a species independent of its closely related forms As a matter of fact, some workers have long looked upon such gelatin-liquefying organisms simply as proteolytic varieties of *Streptococcus fecalis*, while others have considered them to be non-hemolytic strains of *Streptococcus zymogenes*

As to whether or not *Streptococcus liquefaciens*, like *Streptococcus fecalis*, may occasionally be the cause of human infections there is little information, since non-hemolytic streptococci from such sources are not routinely tested for gelatin-liquefying power However, Elser and Thomas (1936) have found that the gelatin-liquefying streptococci recovered in pure culture from the blood in subacute cases of endocarditis are characteristically non-hemolytic

The primary source of *Streptococcus liquefaciens*, as in the case of the other enterococci, is probably the intestines of man and other warm-blooded animals It is widely distributed, however, and its hardy nature equips it for growth under diverse conditions It is frequently found in dairy and other food products in which it is able to grow vigorously This organism has been isolated from plants and some of the strains obtained from this source are much more actively proteolytic than are the characteristic types obtained from stools and milk Based on what is now known, probably as good reasons as any for considering *Streptococcus liquefaciens* as a species independent of its hemolytic

relative, *Streptococcus zymogenes*, are the apparently wider natural occurrence of *Streptococcus liquefaciens*, and the stronger proteolytic action of many of its strains

Streptococcus zymogenes (Lancefield Group D)

MacCallum and Hastings (1899) reported an organism obtained from a case of endocarditis which they described under the name of *Micrococcus zymogenes* They gave a remarkably clear morphological and cultural description of this organism and pointed out its resemblance to *Streptococcus pyogenes* and the pneumococci on the one hand, and to the pyogenic staphylococci on the other The organism was found to liquefy gelatin and to digest casein, and among the other unique characteristics to which attention was called was a strong reducing action in litmus milk, the litmus being completely reduced before coagulation of the milk They also noted that glycerol stimulated the growth of this organism, plainly indicating its utilization Although the hemolysis of blood was not in use as a test at the time of MacCallum and Hastings' work, their description is sufficiently clear and comprehensive to mark rather definitely the type of organism with which they dealt At the present time, the only adequately described streptococci which fulfill entirely the combination of characteristics given by MacCallum and Hastings to their organism are the hemolytic *Streptococcus zymogenes* and the non-hemolytic type which is sometimes separately designated as *Streptococcus liquefaciens*

MacCallum and Hastings indicated that their organism was probably a common intestinal form since types which appeared to be identical had also been isolated from sewage Shortly after the work of MacCallum and Hastings, this organism was found in specimens from human autopsies by Harris and Longcope (1901), and since that time it has been reported occasionally from clinical and fecal sources (Birge, 1905, Hicks, 1912, Torrey, 1926, Frobisher and Denny, 1928, Sherman and Stark, 1931, Torrey and Montu, 1934, Elser and Thomas, 1936, Sherman, Stark and Mauer, 1937)

Although given the generic name *Micrococcus* by MacCallum

and Hastings, this organism was early recognized as belonging to the genus *Streptococcus* (Winslow and Winslow, 1908) and most of the more recent investigators have so classified it

In connection with *Streptococcus zymogenes*, there is little need to review the relatively large body of literature which has accumulated on "hemolytic enterococci" since most of the discussions given in these publications are too indefinite to give an accurate idea of just what organisms were studied Likewise, there are numerous papers which have dealt with hemolytic streptococci obtained from feces which do not give sufficient information to allow one to judge whether or not the organisms isolated were in fact enterococci Doubtless many if not most of these workers dealt with *Streptococcus zymogenes* or a closely related type, but with the exception of a few, such as Weatherall and Dible (1929) and Meyer (1926), it is not safe to draw such a conclusion

In the work of O T Avery and Cullen (1919) and R C Avery (1929a) on hydrogen-ion and methylene-blue tolerance of hemolytic streptococci, there was obtained from cheese a type which was characterized by the production of a lower final pH in glucose broth and a greater tolerance to methylene blue than were human and bovine pathogenic forms Eight of these cultures served as the foundation for the Lancefield (1933) group D, and Lancefield and Hare (1935), who obtained a larger collection of group D streptococci from the human vagina, pointed out that these organisms are in reality more closely related to *Streptococcus fecalis* than to *Streptococcus pyogenes*, thus allying them with the enterococcus group Hare and Maxted (1935) then found group D streptococci in feces and related these to the hemolytic enterococci of previous workers On the basis of physiological studies, Sherman, Stark and Mauer (1937) concluded that the cultures of group D hemolytic streptococci tested by them weie the same as their own cultures of *Streptococcus zymogenes*, and this conclusion has been subsequently verified by the serological classification of a fairly large collection of strains of *Streptococcus zymogenes* isolated from feces and from milk

Although *Streptococcus zymogenes* has always been considered a proteolytic organism, Sherman, Stark and Mauer (1937) concluded that non-proteolytic strains of this organism should be recognized This suggestion was based upon the fact that hemolytic but non-proteolytic strains appeared to be otherwise entirely identical with the proteolytic type The soundness of this view is substantiated by the serological classification of *Streptococcus zymogenes*, proteolytic and non-proteolytic types both falling in the Lancefield group D More recent investigations (Smith and Sherman, 1937, Niven and Sherman, 1937) with collections from human feces and from milk indicate, contrary to former impressions, that the non-proteolytic type is in fact the prevailing one The loss of proteolytic power is well known to occur in the *Proteus* and *Aerobacter* groups, and it requires no fundamental adjustment in our ideas to recognize the occurrence of proteolytic and non-proteolytic strains in the same streptococcus species

As has already been noted, the differentiation of the so-called *Streptococcus liquefaciens* from *Streptococcus zymogenes* may be an artificial separation Although *Streptococcus zymogenes* has usually been designated as hemolytic, several investigators have made no distinction between hemolytic and non-hemolytic types, thus considering *Streptococcus zymogenes* as giving diverse actions on blood Indeed, Elser and Thomas (1936), on the basis of strains obtained from clinical sources, consider the non-hemolytic type as the prevailing form of *Streptococcus zymogenes* As in the case of *Streptococcus mastitidis*, it is entirely reasonable to consider *Streptococcus zymogenes* as a species containing both hemolytic and non-hemolytic forms The loss of hemolytic power in laboratory cultures of other types of streptococci has been reported a number of times, and though most of these observations might be considered somewhat equivocal, the results of Lancefield (1934b), previously referred to, and those of Grinnell (1928) with single-cell cultures are quite convincing

In view of the apparent variability in hemolytic and proteolytic properties of organisms belonging to this general type, Sherman, Stark and Mauer (1937) have suggested that *Strepto-*

coccus zymogenes and *Streptococcus liquefaciens* might be considered simply as varieties of *Streptococcus fecalis*, the general group becoming one species with its several varieties

Streptococcus fecalis (hemolysis −, proteolysis −)
S fecalis var hemolyticus (hemolysis +, proteolysis −)
S fecalis var liquefaciens (hemolysis −, proteolysis +)
S fecalis var zymogenes (hemolysis +, proteolysis +)

However rational such a consolidation might be, it is probable that bacteriologists will continue to classify proteolytic, and hemolytic, streptococci of this group as separate species, and almost certainly when these two characteristics are combined in the same organism As lines between species are now generally drawn in bacterial taxonomy, there is ample precedent as well as convenience in recognizing the proteolytic and hemolytic *Streptococcus zymogenes* as a species type distinct from the non-proteolytic and non-hemolytic *Streptococcus fecalis* Such a differentiation leaves as connecting links, or varieties, those types which are proteolytic but not hemolytic (*Streptococcus liquefaciens*) or hemolytic but not proteolytic If these "connecting links" are not to be considered as species, it is probably more logical to consider them as varieties of *Streptococcus zymogenes* rather than to assign them to *Streptococcus fecalis*, inasmuch as mutation with the loss of characters is more common than the mutation which results in the acquisition of new characters

In considering the close relationship of the members of the enterococcus group to one another, a few words may be said about their serological kinship Lancefield (1937) has shown that the extracts from a few representative strains of *Streptococcus fecalis* and *Streptococcus liquefaciens* react with her group D antisera, thus appearing to belong to group D Following this lead, we have tested more than 20 cultures each of these two organisms against group D sera prepared by the use of *Streptococcus zymogenes* as the immunizing agent All of these cultures gave definite reactions, most of them strong Of some interest is the fact that a number of the strains of *Streptococcus liquefaciens* were strongly proteolytic types isolated from plants, and that these also reacted with the group D sera Sweeping

statements concerning the serological grouping of *Streptococcus fecalis* and *Streptococcus liquefaciens* would not be justified at this juncture, but it does appear on the basis of what is now known that the Lancefield group D, like group B, contains non-hemolytic as well as hemolytic members From the statement which occurs in the abstract of Houston's address (1936) it would appear that somewhat similar results have been obtained by European workers "With Lancefield's technique, Graham showed all enterococci examined to belong to one group"

Streptococcus zymogenes is the most characteristic hemolytic streptococcus of the normal human intestine, isolations made both by direct and selective methods indicating that it is commonly if not usually the predominating hemolytic form It has little or no virulence for laboratory animals, and in spite of its clinical history as an occasional invader of the human body, *Streptococcus zymogenes*, in common with the other enterococci, is to be considered as essentially non-pathogenic On the other hand, the difference between statistical importance and total human importance should be kept in mind Some investigators (Houston, 1934, 1936) are convinced that *Streptococcus zymogenes* and other enterococci should be considered as significant factors in human health

Streptococcus durans

Although not formally classified, *Streptococcus durans* has been known for more than a decade as an organism which is occasionally found in milk and milk products This hemolytic streptococcus was first isolated from a baby food which contained powdered milk and was looked upon by the original investigators as being *Streptococcus pyogenes*, or at least a hemolytic streptococcus of probable importance from the standpoint of human health Although cursory studies of this streptococcus showed that it was physiologically clearly different from the pathogenic types of hemolytic streptococci, and had no virulence for laboratory animals, the confusion caused by its occasional isolation from dairy products led Sherman and Wing (1935, 1937) to study this organism in detail and name it *Streptococcus durans* because of its rather marked tolerance to heat and desiccation

On the basis of its physiological characteristics, *Streptococcus durans* was related by these workers to the enterococci, though it was not then known to be of intestinal origin Recent studies of the hemolytic streptococci of the human intestine (Smith and Sherman, 1937) have shown that this organism commonly occurs in human feces Although a number of species of hemolytic streptococci may be isolated from human feces, so far as present information extends *Streptococcus durans* and *Streptococcus zymogenes* are the only intestinal types which may properly be designated as "hemolytic enterococci"

As is shown by the data contained in tables 1 and 5, *Streptococcus durans*, on physiological grounds, is clearly a member of the enterococcus division of the streptococci, this relationship is made obvious by its temperature limits of growth, heat resistance, and tolerance to salt, alkali and methylene blue In common with the other enterococci, *Streptococcus durans* is bile tolerant, and although it is strongly hemolytic on blood agar plates, its hemolysin is destroyed in broth cultures so that a negative reaction is usually obtained when it is subjected to the conventional test for the formation of "soluble hemolysin" All of the cultures studied by Sherman and Wing hydrolyzed sodium hippurate, and only one of 40 strains was able to ferment sucrose, however, the more recent study of strains obtained from a wider variety of sources has shown that sodium hippurate may or may not be attacked, as is true of the other species of the enterococci, while a slightly larger proportion of strains ferment sucrose than was indicated by the earlier work

Although it would appear that *Streptococcus durans* should unquestionably be classified as an enterococcus, the physiological reactions indicate that it is more distinctly differentiated from either *Streptococcus fecalis* or *Streptococcus zymogenes* than are the latter two organisms from each other From the hemolytic *Streptococcus zymogenes*, *Streptococcus durans* differs in not having such strong reducing action, in never being proteolytic, and in its inability to ferment so many of the test substances, *Streptococcus durans* does not ferment glycerol nor sorbitol and usually does not attack mannitol nor sucrose, whereas *Streptococcus zymogenes* generally ferments all four of these substances

Streptococcus durans has not been serologically classified by the Lancefield method, but preliminary work indicates that it probably belongs to group D *Streptococcus durans* extracts react with strong group D antisera, but not so well as do extracts of *Streptococcus zymogenes* As a successful grouping serum has not yet been prepared with *Streptococcus durans* as the antigen, definite conclusions should not be drawn concerning its relationship to the established Lancefield groups On the basis of these preliminary observations, however, it probably may be safely concluded that *Streptococcus durans* is serologically as well as physiologically closely related to *Streptococcus zymogenes*, and that its classification as a member of the enterococci is sound

Based on present information, *Streptococcus durans* is to be considered simply as an intestinal streptococcus with resistant and tolerant characteristics which permit it, in common with other enterococci, to survive and grow in environments other than its natural habitat Tests with laboratory animals indicate that it is non-pathogenic, it has not been reported from clinical sources and there is no reason for thinking that it has much if any significance from the standpoint of human health

CONCLUDING COMMENT

One cannot conclude an attempt to bring some order out of the systematic relationships of the streptococci without a feeling of chagrin, not to say one of humiliation and futility *Of one* thing we may rest assured If the present attempt does *not at* once appear ridiculous, it will most certainly have that appearance twenty-five years hence The question naturally arises whether or not such efforts as this are worthwhile, if they add to the confusion which exists rather than contributing to progress Regardless of the merits or demerits of any one effort, there can be no doubt that, over a course of years, progress is made in the classification of bacteria And such progress has value quite aside from its taxonomic aspects Those who have the least interest in the classification of bacteria, in a formal sense, are still engrossed in the differentiation of closely related microorganisms, by one means or another, for the attainment of practical ends

Many workers have struggled with the question of what constitutes a species among bacteria, or even whether such a unit can be defined. The statement of Andrewes (1906), now more than thirty years old, is still good; some of it may seem a little trite at this late date, but his idea about variability in "dominant genera" perhaps still has pertinence in connection with the streptococci.

It may very properly be asked whether the attempt to define distinct species, of a more or less permanent nature, such as we are accustomed to deal with amongst the higher plants and animals, is not altogether illusory amongst such lowly organised forms of life as the bacteria. No biologist nowadays believes in the absolute fixity of species. It is recognised that those groups of like individuals, which we agree to group together for our convenience under a common name, have arisen from pre-existing species and that in many cases transitional forms can be found. It has further been made plain that the relative fixity of specific distinctions varies widely in different groups. Those forms of life which are manifestly succeeding above their fellows in the struggle for existence and are rapidly adapting themselves to new environments, show, as a rule, less specific fixity than other forms. The bounds of the individual species are ill-defined and transitional forms are numerous. Amongst British plants the brambles are often quoted as such a group; botanists have long been puzzled to define the species and have been compelled to resort to the idea of sub-species. Hooker describes no less than 21 sub-species of the common blackberry.

Such successful and variable groups are often spoken of as "dominant genera" and they are to be found as much amongst the bacteria as higher in the vegetable scale. But there are two circumstances which here render the problem of specificity even more difficult of solution. The bacteriologist is deprived of the test of mutual fertility or sterility, so valuable in determining specific limits amongst organisms in which sexual reproduction prevails. Further, the extreme rapidity with which generation succeeds generation amongst bacteria offers to the forces of variation and natural selection a field for their operation wholly unparalleled amongst higher forms of life. The machinery for the production of new varieties is enormous in the case of organisms which can provide 20 or 30 generations in a day. We might almost expect the limits of species to be more intangible amongst the bacteria than amongst higher organisms. Nevertheless, many species of bacteria exhibit characters which seem quite fixed and rigid. The anthrax

bacillus and the tetanus bacillus are quite as good "species," in the natural history sense, as any that can be found amongst flowering plants It is only when we come to the dominant genera that difficulties arise The "bacillus coli group" is an excellent example of such a genus The more this group is studied the more perplexing is the maze of species and sub-species, and it is significant that the tests which now enable us to pick out the chief forms are physiological and not morphological

Now what is true of the bacillus coli group is true of the streptococci, which also form a dominant genus Their vast abundance in nature is evidence that they have succeeded in the struggle for existence and are still maintaining their supremacy in the field which they have adapted themselves to fill Their varieties are even more bewildering than those of the bacillus coli group and, as Gordon has shown, they can be differentiated by their physiological powers far better than by morphological structure

The fact is that bacterial classification is still on a statistical basis—the old mountain range, mountain peak theory of species One would think that with the passage of time, if progress is made, the species might be visualized not as peaks in a mountain range, but more like trees in a fence row connected by the inevitable underbrush Such progress has been made, albeit the "underbrush" still bulks large One cannot believe in evolution without believing in variation, hence the "intermediates" will long remain with us

In a whimsical mood and quite extemporaneously, Justice Oliver Wendell Holmes once remarked

Facts in isolation amount to mere gossip, facts in relation become philosophy

And this, perhaps, gives the key to one of the chief ailments of bacterial taxonomy There are too many "*facts in isolation*" and too few "facts in relation" In the laudable effort to find a few definitive tests, there has been too much tendency to center on those which are considered good, at the same time discarding others which may be of greater value in *dealing* with the members of closely related species When a test is found of value in

studying certain types of streptococci it is proper to apply it to all others—but not at the expense of other reactions which may, in spite of their apparent futility, have special functions when applied to new groups. Trehalose, for example, has outstanding value in its application to the streptococci belonging to the Lancefield group C, but with the streptococci as a whole, exclusive of group C, it is not apparent that its usefulness is greater than that of maltose or sucrose. Maltose and sucrose have little general utility in the study of streptococci and have been very generally discarded. But maltose becomes quite pertinent when one is concerned with *Streptococcus thermophilus*, and also has a limited usefulness in the lactic group. Sucrose has a distinct statistical value among the streptococci belonging to the lactic and enterococcus divisions. It is recognized in other fields of biology that characters which have great differential value within one group may be quite worthless in another, but in bacteriology we have fallen prey, to a greater extent, to the fetish of standard methods and uniform charts.

It is not intended to give the impression that practical workers should apply every known test for the identification of each streptococcus studied, but for the final clarification of the various species, use will have to be made of all present methods, and of new ones yet to be developed. From such studies will evolve, eventually, the few incisive reactions needed for the identification of each species. In the meantime, imperfect or "majority" tests have distinct value in revealing the statistical pattern. As the Winslows (1908) have stated:

> If the same strains are considered statistically, that is if the *frequency* of a given character be taken into account, it is apparent that certain combinations of characters are much more common than others. Measurement of almost any character by quantitative methods shows that the bacteria examined group themselves on a simple or complex curve of frequency. The modes of this curve indicate centers of variation about which the individuals fluctuate, and these centers of variation are the real systematic units of the group. The recognition of such centers, as specific types, offers the natural and satisfactory compromise between systematic multiplicity and vague generalization.

Although we are now resigned to the conclusion that probably no one physiological reaction is quite infallible, with the employment of a large number of them the better known species of the streptococci can be identified with a very high percentage of accuracy But barring those groups which are amenable to the Lancefield technique, no comparable accuracy can yet be attained with the few tests which are commonly used in the study of these organisms And of great importance is the fact that when streptococci are studied by means of a broad list of reactions, individual strains which are atypical with respect to some character which is normally most constant can still be identified with a considerable degree of assurance

One naturally wonders if the goal of a simple and positive identification of all streptococcal species will be reached with serological methods Further brilliant achievements will doubtless issue from the immunological approach, but too much should not be expected in the near future As further use is made of the antigenic complex of organisms, the unity of living matter, as well as its diversity, becomes important The possibilities are both manifold and complex To illustrate, a few examples may be cited Bliss (1937) has shown that serological type I in the Lancefield group F and type I in group G have an identical type antigen, the type II pneumococcus and one variety of the Friedlander bacillus appear to have the same soluble specific substance (Avery, Heidelberger and Goebel, 1925), and Kendall, Heidelberger and Dawson (1937) have isolated a serologically inactive polysaccharide, from mucoid strains of *Streptococcus pyogenes*, which appears to be chemically identical with that occurring in bovine vitreous humor and human umbilical cord

But the future will doubtless take care of itself—and most probably in a manner not now anticipated As for this review, if it helps to bring just a few facts "in relation" the effort will be justified, but the systematization of the streptococci is still far from a "philosophy"

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